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Effect of Mesh Size on Selectivity of Square Mesh Codends

K.K. Kunjipalu, B. Meenakumari, T. Joseph Mathai, M.R. Boopendranath and R.S. Manoharadoss
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The effect of mesh size on the selective escapement of length class of trawl resources including nine fin fishes and three shell fishes was studied using 20, 25, 30 and 40 mm square mesh codends. Comparatively more juveniles and sub adults were retained in 20 mm square mesh cod ends while 40 mm square mesh permitted escapement of many commercially important groups such as penaeid prawns and squid. 30 mm square mesh cod end has shown compromise size selection properties by providing escapement of most of the juveniles while retaining bulk of economically important species groups. Selectivity parameters and selection curves were determined in respect of twelve species selected for the study.

Key words: Codend selectivity, square mesh, trawl

Pope (1966) has reported that the size and shape of codend meshes determine the selectivity of codend in trawls. Gulland (1969) suggested a method to find out the mean selection length (L_{50}) which is proportional to the mesh size (mm) of codend of trawl. The selectivity of square mesh codends was studied by Robertson (1982, 1983a,b; 1984, 1986), Robertson & Stewart (1986), Kunjipalu *et al.* (1994a,b; 1997), Varghese *et al.* (1996) and Pillai *et al.* (1996). The present study was undertaken to find out the effectiveness of four mesh sizes in square mesh codends in facilitating escapement of juveniles.

Materials and Methods

Data for the study were collected from experiments carried out with covered codends, conducted during July 1995-June 1997, off Cochin, Kerala. Four square mesh codends with 20, 25 30 and 40 mm mesh size were fabricated and fitted with covers of small mesh size of 10 mm as described by Kunjipalu *et al.* (1994a,b). Experimental square mesh codends were operated with semi balloon trawls and demersal trawls described by Kunjipalu *et al.* (1998a,b). Fishes collected in the cover and inner codend were sorted out species-wise and length statistics were taken for determining percentage escapement. Selectivity parameters and selection curves in respect of different species were determined as described by Sparre *et al.* (1969).

Results and Discussion

Percentage escapement of different length classes of 9 fin fish resources viz. *Lactarius* sp., *Johnius* sp., *Leiognathus* sp., *Dussumieria* sp. *Caranx* sp., *Stolephorus* sp. *Thryssa* sp., *Cynoglossus* sp., *Decapterus* sp. and three shellfish resources viz. *Metapenaeus dobsoni, Parapenaeopsis stylifera* and *Loligo* sp. are given in Tables 1-12, separately for 20, 25, 30 and 40 mm codends. Selectivity curves and estimated selectivity parameters in respect of these species for square mesh codend are given in Figs. 1-12 and Table 13, respectively.

As seen from Tables escapement of juveniles and sub-adults of all species was found to be less from 20 mm square mesh codend compared to the other three mesh sizes. On the contrary, 40 and 30 mm square

Table 1. Percentage escapement of different length groups of *Lactarius lactarius* in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	59.00	100.00	100.00	100.00
40-59	76.61	100.00	97.21	100.00
60-79	46.74	27.59	90.96	100.00
80-99	0.00	6.75	52.83	100.00
100-119	0.00	0.94	0.00	0.00
120-139	0.00	0.00	0.00	0.00
140-159	0.00	0.00	0.00	0.00
160-179	0.00	0.00	0.00	0.00
180-199	0.00	0.00	0.00	0.00

Table 2. Percentage escapement of different length groups of *Johnius* sp. in 20, 25, 30, and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	59.09	100.00	100.00	100.00
40-59	58.24	79.66	96.35	100.00
60-79	19.95	38.79	83.33	98.36
80-99	2.15	3.08	16.56	58.79
100-119	0.07	0.50	0.17	32.81
120-139	0.00	0.00	0.00	5.60
140-159	0.00	0.00	0.00	0.00
160-179	0.00	0.00	0.00	0.00
180-199	0.00	0.00	0.00	0.00
200-219	0.00	0.00	0.00	0.00
220-239	0.00	0.00	0.00	0.00
240-259	-	0.00	0.00	0.00

Table 3. Percentage escapement of different length groups of *Leiognathus* sp. in 20, 25, 30 and 40 mm square mesh codents

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	98.00	91.27	100.00	100.0
40-59	53.83	64.58	93.85	100.0
60-79	10.81	13.21	43.97	93.5
80-99	1.50	0.00	3.33	1.85
100-119	0.00	0.00	0.00	0.00
120-139	0.00	0.00	0.00	0.00
140-159	0.00	0.00	0.00	0.00

Table 4. Percentage escapement of different length groups of *Dussumieria acuta* in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	0.00	0.00	0.00	0.00
40-59	0.00	0.00	0.00	0.00
60-79	54.22	100.00	100.00	100.00
80-99	21.85	76.06	61.52	100.00
100-119	8.44	27.40	38.26	100.00
120-139	0.00	0.96	1.02	74.16
140-159	0.00	0.35	0.00	0.00
160-179	0.00	0.00	0.00	0.00
180-199	0.00	0.00	0.00	0.00
200-219	0.00	0.00	0.00	0.00

Table 5. Percentage escapement of different length groups of *Caranx para* in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	0.00	0.00	100.00	100.00
40-59	96.32	0.00	94.29	100.00
60-79	13.70	100.00	53.95	85.96
80-99	21.07	17.46	25.14	50.84
100-119	0.00	2.27	10.41	31.89
120-139	0.00	0.00	0.50	4.38
140-159	0.00	0.00	0.00	0.00
160-179	0.00	0.00	0.00	0.00

Table 6. Percentage escapement of different length groups of *Stolephorus* sp. in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	99.70	98.96	100.00	100.00
40-59	92.40	95.75	94.95	100.00
60-79	80.01	84.02	79.88	99.81
80-99	87.00	79.78	89.33	97.70
100-119	0.00	22.54	4.92	75.00
120-139	0.00	0.00	0.00	0.00

Table 7. Percentage escapement of different length groups of *Thryssa* sp. in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	100.00	100.00	100.00	100.00
40-59	100.00	100.00	100.00	100.00
60-79	80.20	67.52	92.09	100.00
80-99	41.83	16.40	66.34	100.00
100-119	4.61	5.88	55.98	90.00
120-139	0.00	0.00	0.00	6.25
140-159	0.00	0.00	0.00	0.00
160-179	0.00	0.00	0.00	0.00
180-199	0.00	0.00	0.00	0.00

Table 8. Percentage escapement of different length groups of *Cynoglossus* sp. in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
60-79	92.45	100.00	100.00	100.00
80-99	63.43	100.00	87.05	100.00
100-119	49.35	3.05	42.01	100.00
120-139	0.00	0.00	30.77	79.02
140-159	0.00	0.00	0.00	0.00
160-179	0.00	0.00	0.00	0.00
180-199	0.00	0.00	0.00	0.00

Table 9. Percentage escapement of different length groups of *Decapterus* sp. in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
40-59	100.00	100.00	100.00	100.00
60-79	100.00	100.00	100.00	100.00
80-99	82.22	100.00	99.15	92.89
100-119	0.00	11.00	64.71	51.72
120-139	0.00	6.00	0.00	52.94
140-159	0.00	0.00	0.00	0.00
160-179	0.00	0.00	0.00	0.00
180-199	0.00	0.00	0.00	0.00
200-219	0.00	0.00	0.00	0.00

Table 10. Percentage escapement of different length groups of *Metapenaeus dobsoni* in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	96.16	100.00	100.00	100.00
40.59	42.30	51.13	73.28	100.00
60.79	3.96	19.55	51.00	29.73
80-99	0.30	0.00	1.02	12.23
100-119	0.00	0.00	0.00	0.00

Table 11. Percentage escapement of different length groups of *Parapenaeopsis stylifera* in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	91.67	87.02	100.00	100.00
40-59	73.58	67.58	82.01	100.00
60-79	15.46	7.76	43.51	31.00
80-99	0.14	1.36	2.91	9.00
100-119	0.00	0.00	0.00	0.00

Table 12. Percentage escapement of different length groups of *Loligo* sp. in 20, 25, 30 and 40 mm square mesh codends

Length group (mm)	Escapement			
	20 mm	25 mm	30 mm	40 mm
20-39	89.60	94.67	98.78	100.00
40-59	45.08	54.17	81.91	100.00
60-79	0.00	0.00	34.45	76.40
80-99	0.00	0.00	0.00	34.78
100-119	0.00	0.00	0.00	7.41
120-139	0.00	0.00	0.00	0.00
140-159	-	0.00	0.00	0.00
160-179	-	0.00	0.00	0.00
180-199	-	0.00	0.00	0.00
200-219	-	0.00	0.00	0.00
220-239	-	-	0.00	0.00
240-259	-	-	-	0.00

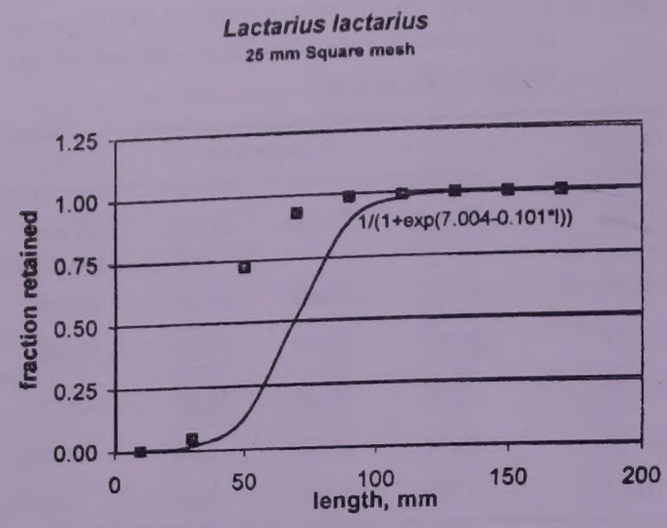

Fig. 1. Selectivity curve for *Lactarius lactarius*

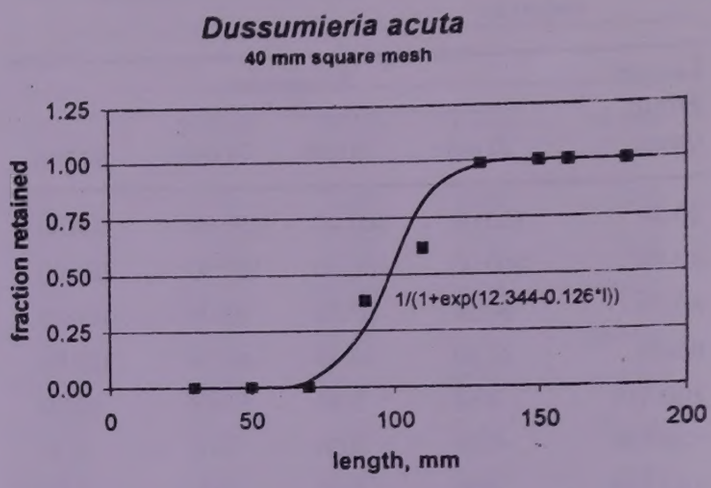

Fig. 4. Selectivity curve for *Dussumieria acuta*

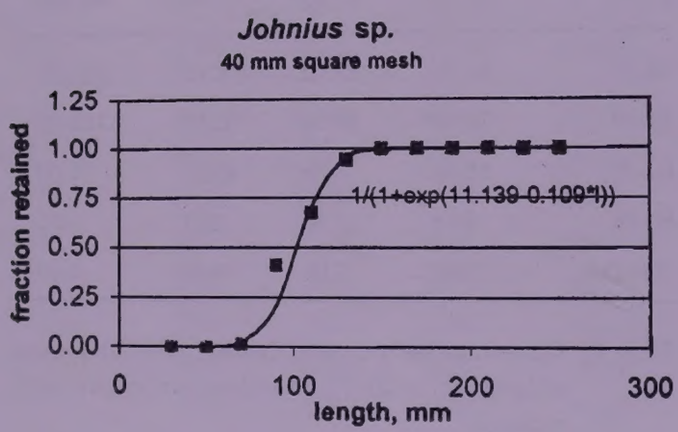

Fig. 2. Selectivity curve for *Johnius* sp.

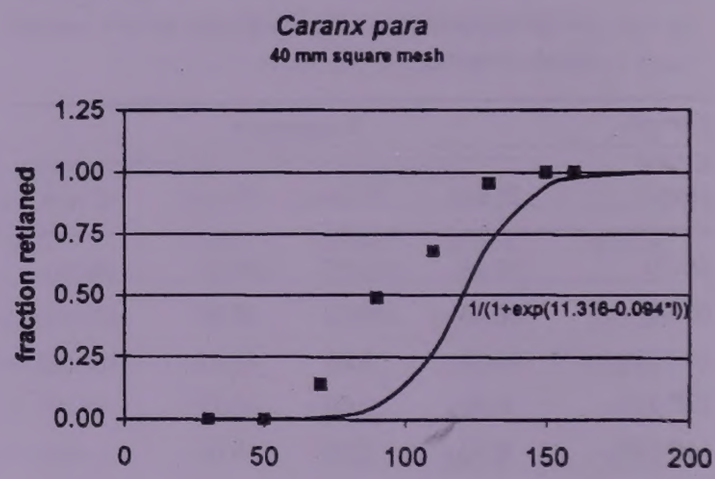

Fig. 5. Selectivity curve for *Caranx para*

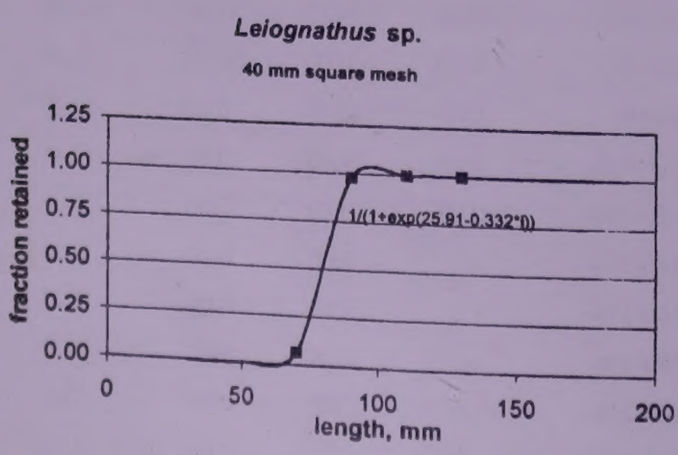

Fig. 3. Selectivity curve for *Leiognathus* sp.

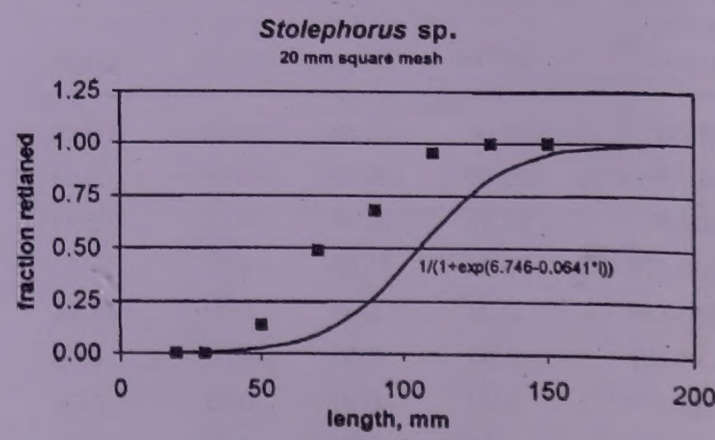

Fig. 6. Selectivity curve for *Stolephorus* sp.

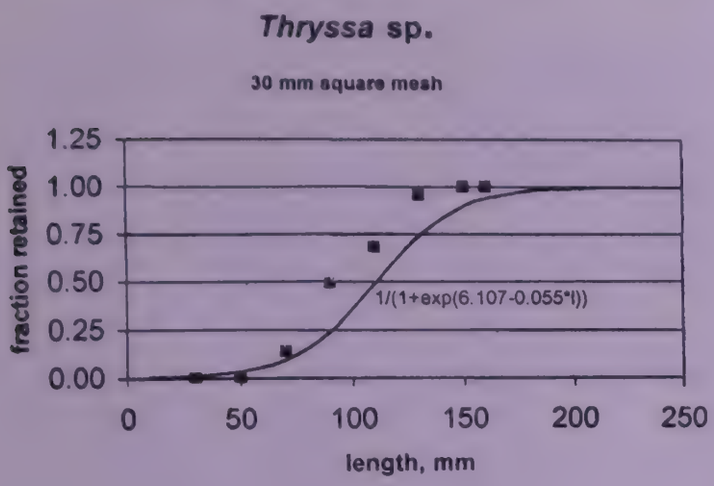

Fig. 7. Selectivity curve for *Thryssa* sp.

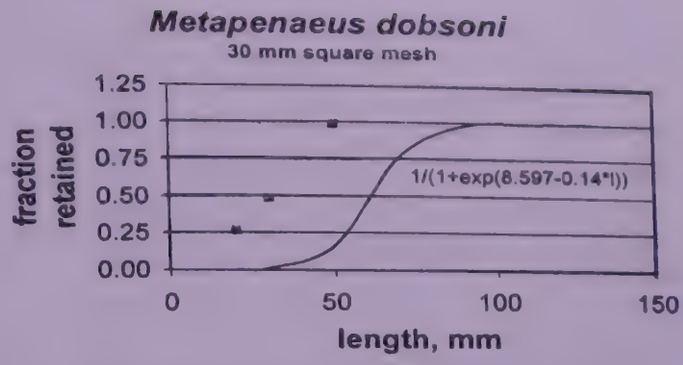

Fig. 10. Selectivity curve for *Metapenaeus dobsoni*

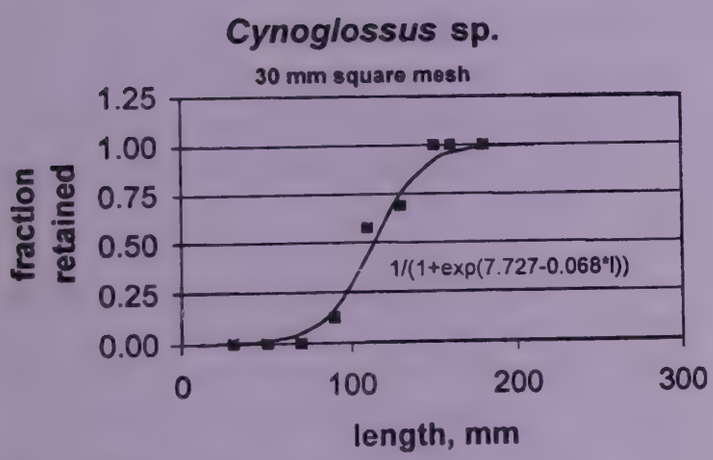

Fig. 8. Selectivity curve for *Cynoglossus* sp.

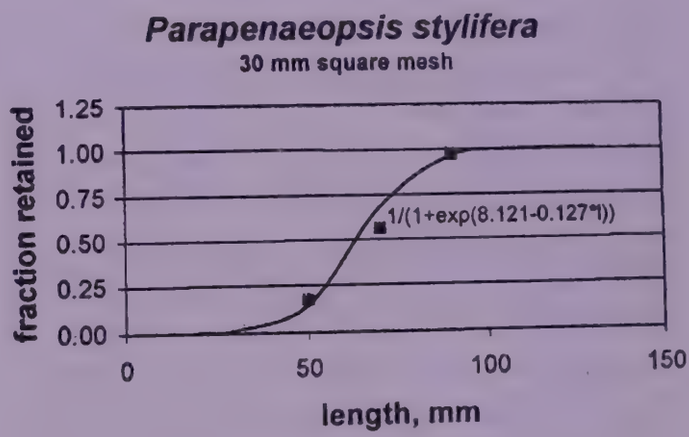

Fig. 11. Selectivity curve for *Parapenaeopsis stylifera*

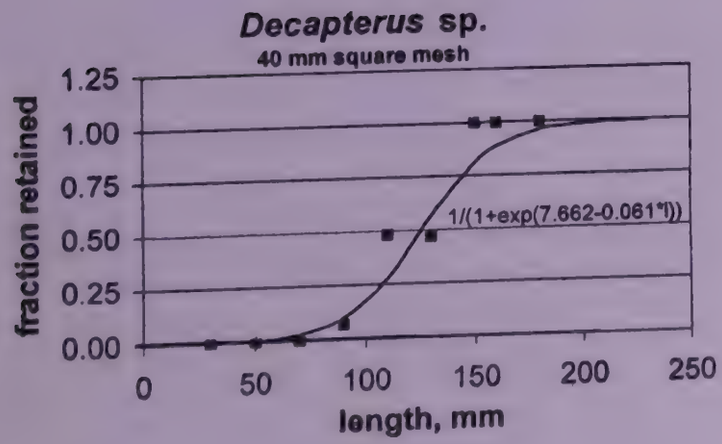

Fig. 9. Selectivity curve for *Decapterus* sp.

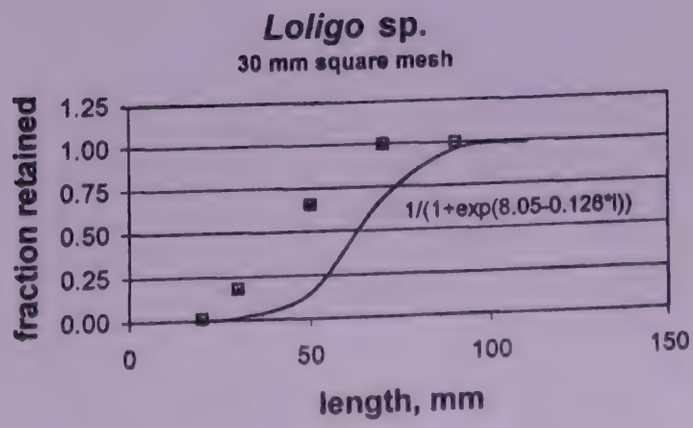

Fig. 12. Selectivity curve for *Loligo* sp.

Table 13. Selectivity parameters for square mesh codend

Species	Mesh size, mm	$L_{25\%}$ mm	$L_{50\%}$ mm	$L_{75\%}$ mm	Selection range, mm	Selection factor
L. lactarius	25	58.47	69.35	80.22	21.75	2.77
Johnius sp.	40	92.11	102.19	112.27	20.16	2.56
Leiognathus sp	40	74.73	78.04	81.35	6.62	1.95
D. acuta	30	89.25	97.97	106.69	17.44	3.27
C. para	40	108.70	120.38	132.07	23.37	3.01
Stolephorus sp.	20	88.24	105.41	122.57	34.33	5.27
Thryssa sp.	30	91.06	111.04	131.01	39.95	3.70
Cynoglossus sp.	30	90.86	107.01	123.17	32.31	3.57
Decapterus sp.	40	107.60	125.61	143.62	36.02	3.14
M. dobsomi	30	53.56	61.41	69.25	15.69	2.05
P. stylifera	30	55.29	63.94	72.60	17.30	2.13
Loligo sp.	30	55.17	63.89	72.61	17.44	2.13

mesh codends provided 100 percent or near 100 percent escapement of juveniles of all species considered for the study. 25 mm square mesh codend also facilitated the escapement of a certain percentage of juveniles next to 30 mm square mesh codend. However, 40 mm square mesh codend permitted escapement of some quantity of commercial size groups of economically important fishes especially penaeid prawns (*M. dobsoni* and *P. stylifera*) Tables 10 and 11) and squid (*Loligo* sp.) (Table 12).

In the case of *Lactarius lactarius*, 100% escapement was observed in the size group of 80-99 mm from 40 mm square mesh codend (Table 1). In the case of *Johnius* sp. (Table 2), escapement of size groups 80-99mm was 58.79% and of 100-119 mm it was 32.81% from 40 mm square mesh codend. On the other hand, escapement of juveniles and sub-adults was in the range of 58.24-59.09% in 20 mm square mesh codend, while it was 79.66-100% in 25 mm, 96.35-100% in30 mm and 100% in 40 mm square mesh codend (Table 2).

As can be seen from Tables 10, and 11, 29.73% of 60-79 mm size group of *M. dobsoni* and 31% of *P. stylifera* escaped from 40 mm square mesh codend. Also, 34.78% of 80-90 mm size and 76.40% of 60-79 mm size groups of *Loligo* sp. escaped from 40 mm square mesh codend (Table 12).

It is evident from the analysis that mesh size below 25 mm in square mesh codend provided very little chance of escapement for juveniles; whereas mesh size 30 mm provided better chance of escapement of juveniles and sub-adults. Hence, minimum mesh size is suggested to be above 25 mm and preferably 30 mm for square mesh codends in demersal trawls operated in the inshore waters for better management and conservation of fish stocks. Kunjipalu *et al.* (1997) have already reported that the size of mesh is more effective than the shape of mesh whether diamond or square, especially when the size of mesh is as small as 20 mm.

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Radiation Technology for control of *Listeria monocytogenes* and *Yersinia enterocolitica* in Fish

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The efficacy of the radicidation as a method of preservation of fish was evaluated by artificially inoculating *Listeria monocytogenes* or *Yersinia enterocolitica* cells prior to irradiation and detecting the respective pathogen during storage at 2-4°C. These studies when repeated with two serotypes of *L. monocytogenes* clearly suggested the need of a dose of 3 kGy for elimination of 103 cfu.g^{-1} from air packed frozen shrimp. In case of *Y. enterocolitica*, a dose of 1 kGy was sufficient to eliminate naturally occurring low numbers from shrimp without any revival during storage at chilled temperature for two weeks. The initial level of contamination was an important factor in deciding the dose of radiation for effective control of growth of these organisms.

Key words : Gamma irradiation, *Listeria monocytogenes*, *Yersinia enterocolitica*,

Yersinia enterocolitica and Listeria monocytogenes are potential pathogens having occurrence in a variety of foods and meat (Farber & Peterkin, 1991, Palumbo, 1986). The organisms, being psychrotrophs, can grow in foods at refrigeration temperature and cause food borne diseases. The major outbreaks of listeriosis and yersiniosis in different parts of the world were associated with consumption of foods of animal origin. This has led to increased concern world wide for detecting these organisms in the foods and investigating means to eliminate them. Many studies conducted over 40 years have revealed irradiation as one of the cost-effective technologies in processing wholesome and safe foods (Farkas, 1990; Radomysky *et al.*, 1994; Anon, 1993). One advantage of this technology is that irradiation, being a nonthermal process, can bring about extension of shelf life of raw fish/meat at refrigeration temperature and help in marketing the product as chilled (0 - 10°C) instead of frozen, thus meeting the demand for fresh foods. Reports from our laboratory (Kamat *et al.*, 1991; Kamat & Nair, 1995; Kamat *et al.*, 1997) as well as from elsewhere (Diehl, 1994 and Radomysky *et al.*, 1994) have demonstrated that gamma irradiation in the range of 2-7 kGy, depending upon the level of natural contamination and irradiation temperature, eliminated many hazardous food borne pathogens from sea food and made the product microbiologically safe. The results of the work carried out to establish the minimum radiation dose to decontaminate fish product from *Listeria monocytogenes* and *Yersinia enterocolitica* are presented in this paper

Materials and Methods

L. monocytogenes and other strains of *Listeria*; as well as *Y. enterocolitica* and other strains of *Yersinia* used in the studies were obtained from Public Health Laboratory Services, U.K.

Fresh, frozen and dry varieties of fish from local market were collected in sterile

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plastic bags. The fresh and frozen fish samples were transported to the laboratory in boxes containing ice or dry ice. Ten grams of sample were aseptically added to 90 ml sterile BHI (brain heart infusion medium) and homogenized. Two stage enrichment procedure, including primary cold enrichment in BHI followed by secondary enrichment in selective medium was adopted for isolation of *Listeria* (Kamat & Nair, 1994) and *Yersinia* (Khare *et al.*, 1996). The identification of the pathogens to species level was done on the basis of biochemical tests by comparing with reactions of standard type cultures.

Overnight grown cells of the required cultures *(L. monocytogenes* or *Y. enterocolitica)* were washed with phosphate buffer (pH 7.00). The shrimp samples were completely submerged in cell suspensions at a concentration of 10^3 - 10^4 organisms/ml. After 1 h at 0°C the samples were drained, packed aseptically in sterile polyethylene bags and frozen at -40°C prior to irradiation.

Shrimp samples packed in polyethylene bags were exposed to 1,3,4 and 6 kGy doses in a Co^{60} food package irradiator (Atomic Energy Canada) at a rate of 0.05 $kGy.min^{-1}$ (IAEA, 1977). Unirradiated products served as controls. All the samples were stored at 2 - 4°C in separate containers and periodically checked for the concentration of the inoculated organism.

Table 1. Incidence of *Listeria* spp. in fish and shellfish

Product	No. of samples	No. of isolates	Number of isolates of						
			L. monocytogenes	*L. ivanovii*	*L. innocua*	*L. seeligeri*	*L. murrayi*	*L. grayi*	Un identified
Frozen									
Shrimp	4	65	-	-	21	10	-	34	-
Fresh fish									
Dhoma	3	5	-	2	-	-	-	3	-
Anchovy	2	5	-	-	-	-	3	2	-
Golden anchovy	2	10	-	-	2	-	3	4	1
Pomfret	2	10	-	-	2	-	3	5	-
Indian Salmon	1	5	-	-	-	2	-	1	2
Threadfin (Raja Rani)	3	15	-	-	5	-	5	2	3
Mackerel	3	10	-	-	4	-	-	2	4
Bombay duck	2	10	-	-	-	-	4	4	2
Crab	2	15	-	2	3	1	2	2	5
Dry fish									
Shrimp	3	10	-	2	4	-	2	2	-
Mackerel	2	8	-	-	-	-	-	6	2
Bombay duck	3	14	-	-	8	-	-	4	2
Golden Anchovy	2	10	-	-	2	-	-	6	2
Anchovy	3	9	-	-	-	-	-	9	-
Total	38	201	-	6	51	13	22	86	23

1. Many of the isolates remained unidentified but they remain nonhaemolytic
2. Many isolates belonging to nonhaemolytic *L. innocua* ferment rhamnose

Table 2. Profile of *Yersinia* species isolated from fish

Product	No. of samples	CIN (count g^{-1})	Total number of isolates	Number of *Y. intermedia* isolates	Number of *Y. pestis* isolates	Number of *Y. pseudotuber-culosis* isolates	Number of *Y. enterocolitica* isolates	Number of *Y. fredriksenii* Isolates	Number of unidentified (Mannitol negative) isolates
Fresh finfish									
Anchovy	2	$7x10^4$	6	4(66.6%)	-	-	-	-	2
Bombay duck	6	$3x10^6$	14	8(57.14%)	-	-	-	-	6
Mackerel	5	$2.5x10^5$	8	8(100%)	-	-	-	-	-
Pomfret	1	$6x10^3$	7	4(57.14%)	-	-	1(14,28%)	-	2
Total	14		35	24	-	-	1	-	10
Fresh shellfish									
Shrimp - 1	1	$6x10^4$	18	5(27.77%)	1(5.5%)	1(5.5%)	-	1(5.5%)	10
Shrimp - 2	2	$1.2x10^5$	18	-	-	-	-	-	-
Crab	2	$1.5x10^5$	10	2(20%)	-	-	2(20%)	-	6
Oyster	5	$3.5x10^4$	5	-	-	-	1(20%)	-	4
Prawn	3	$2.5x10^4$	14	3(21.42%)	-	-	-	-	11
Total	12		47	10	1	1	3	1	31
Dried fish and shellfish									
Golden anchovy	5	$2x10^5$	5	3(60%)	-	-	-	-	2
Anchovy	5	$5x10^4$	10	4(40%)	4(40%)	-	1(10%)	-	1
Mackerel	3	$4x10^5$	6	5(83.33%)	-	-	-	-	1
Shrimp	3	$2.2x10^6$	11	10(90.90%)	-	-	-	-	1
Prawn	3	$5.1x10^5$	11	9(81.1%)	-	-	1(9.01%)	-	1
Bombay duck	5	$2.5x10^4$	13	9(69.23%)	-	-	-	-	4
Total	24		56	40	4	-	2	-	10

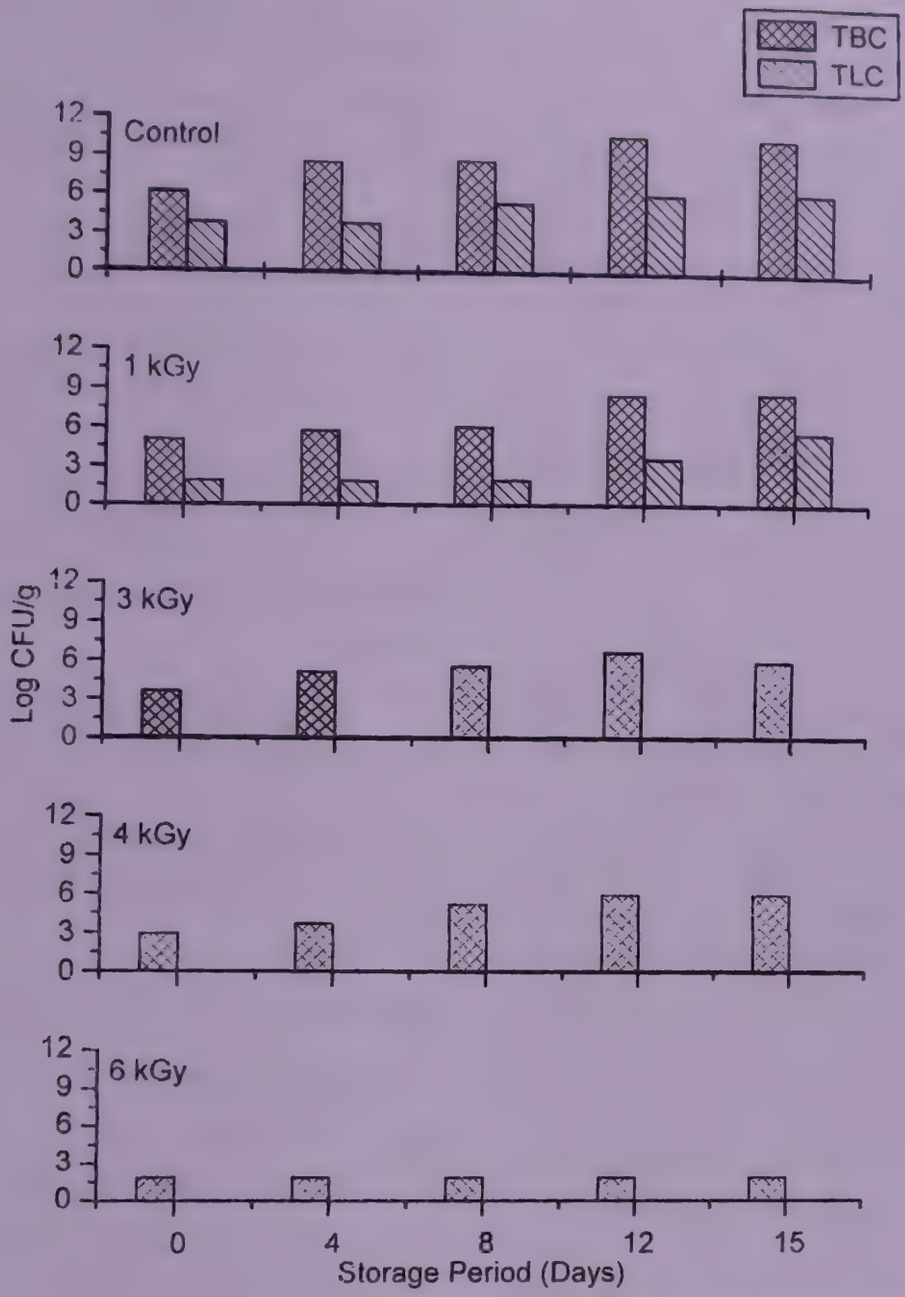

Fig. 1. *L. monocytogenes* in irradiated and unirradiated inoculated fish during storage at 2 - 4°C

The packages were opened periodically, and 10 g of the product were aseptically transferred to 90 ml sterile saline and homogenized for 3 min. Serially diluted shrimp samples were plated in triplicate on the plate count agar (PCA) and respective selective enrichment medium for detection of *L. monocytogenes* or *Y. enterocolitica*.

Results and Discussion

Data on incidence of Listeria in 38 samples of fish are given in Table 1. Out of 38 fish samples, 10 were contaminated with *L. innocua* and 7 samples with *L. murrayi* while *L. grayi* was detected in all samples. However *L. monocytogenes* was not found in any of the samples tested. The findings are in agreement with the only report regarding incidence of this organism in Indian fishery products by Fuchs & Surendran (1989) who examined the incidence of Listeria in local markets of Cochin. However, later it was reported that the recovery of *L. monocytogenes* from Indian fish is possible by modifying the isolation procedures (Jayasekaran *et al.*, 1996).

Table 2 gives the details of *Yersinia* species isolated from fish samples on Cefsiodin-Irgasan - Novobiocin (CIN) agar plates and identified to species level. Out of 138 *Yersinia* isolates recovered from 15 samples, 74 isolates belonged to *Y. intermedia*. Thus the predominant species was non-pathogenic in nature. Nevertheless, it can be seen that seafood like pomfret (14.28%), oyster and crab (20% each) and some dry fish (10%) harboured *Y. enterocolitica*. These findings of incidence of *Y. enterocolitica* in fish are similar to the reports from Canada (Toma, 1973), USA (Lee, 1977) and other parts of the world (Gerard & Leland, 1992).

The D_{10} values of *L. monocytogenes* and *Y. enterocolitica* in fish homogenate at 40°C was observed to be 0.25 - 0.3 kGy (Kamat & Nair, 1992). It is evident from Fig.1 that the initial total bacterial count (TBC) increased to 10^9 cfu. g^{-1} in the case of the control and 1 kGy samples after 4 and 12 days of storage respectively. In contrast, the growth of *L. monocytogenes* was comparatively slow in control and 1 kGy samples which could be due to presence of fast growing organisms surviving in 1 kGy samples (82 cfu. g^{-1}). Shelef (1989) has reported poor growth of *L. monocytogenes*, ATCC 35152, in ground beef in presence of fast growing bacteria. Listeria showed growth to $5.3x10^3$ and 7.0×10^3 in 12 and 15 days, respectively in control and 1 kGy samples and irradiation resulted in lag of 8 days as against 4 days in unirradiated samples. However, at doses 3 kGy and above Listeria were completely eliminated and its revival was not noticed during storage for 15 days. The storage studies of samples artificially inoculated with *Y. enterocolitica* showed the ability of this organism to revive in inoculated fish during post irradiation storage (Table 3). However, this phenomenon was not noticed when naturally contaminated fish was irradiated and stored at chilled temperature, suggesting the significance of initial level of contamination in determining the optimum dose for radiation.

From the results of this investigation it can be safely concluded that irradiation at a dose of 3 kGy, efficiently eliminates *L. monocytogenes* and *Y. enterocolitica* from frozen fish and makes it safe for consumption.

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Table 3. Fate of *Yersinia enterocolitica* in naturally contaminated and inoculated fish during irradiation and storage at 2 - 4°C.

Irradiation dose (kGy.min^{-1})	Storage time (Weeks) Natural				Storage time (Weeks) Inculated (10^6 cell.g^{-1})			
	0	2	3	4	0	1	2	3
0 (Control)	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+
3	+	+	-	-	-	+	+	+
4	-	-	-	-	-	+	+	+
6	-	-	-	-	-	+	+	+

+ = detected; - = not detected

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Physicochemical Properties of Malaysian Fish Balls

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A total of 10 brands of Malaysian fish balls were analyzed for physiochemical properties like colour, cooking effect, proximate composition and texture (folding test). Results showed that there was a wide variation in the amount and types of ingredients used in producing the different kinds of fish balls. The weight of samples analyzed ranged between 6.25 - 15.14 g with price varying from US $ 1.97-3.62/kg. The colour value for lightness (L) of fish ball varied significantly ($p<0.05$), ranging from 62.14-76.85. Different brands of fish balls exhibited different cooking effect, which ranged between -11.06% and +8.21%. All samples showed AA grade in folding test, which indicated that the texture of fish balls was acceptable to consumer.

Key words: Fish balls, proximate composition, folding test

Fish ball is a popular fish based processed product, commonly consumed with noodles in the Southeast Asian region. Fish balls are also popular in many Southeast Asian countries in different local names such as *Bebola* in Malaysia and Brunei, *Bakso* in Indonesia, *Bola-bola* in Philippines, *Jbi-ei* in Singapore and *Luk-chin pla* in Thailand (Chng *et al.*, 1991). Fish ball production used to be usually a small, family-based enterprise. But in recent years, many factories have invested in modern machinery to increase the production of fish balls. The annual production in Malaysia increased from 4,381 t in 1990 to 7,874 t in 1996 (Department of Fisheries, 1996).

The basic ingredients of fish balls are fish meat, starch, salt, sugar and water. The ingredients are usually mixed together thoroughly using mixer, transformed into the shape of balls and then allowed to set in water at 40°C for 20 min, followed at 90°C for 20 min. The fish balls are normally sold fresh or as chilled/frozen in supermarkets (Yu & Yeang, 1993). Attributes such as white colour, absence of fish smell and soft but elastic texture are the desirable qualities of fish balls (Yamprayoon *et al.*, 1991). This study is aimed at providing more information on the physicochemical properties of fish balls, which are produced and marketed in Malaysia.

Materials and Methods

Fish balls from ten producers (Sajimas, Kuantan, Top 1, Armiya, Parang AA, DFI, SIA Parang, GAN, SIA Putih and without brand) from different regions of Malaysia were obtained from local markets and supermarkets. Samples were randomly labelled as A,B,C,D,E,F,G,H,I and J (not according to the brand's order). Two packets of each brand were obtained for analysis. Label information on the package with ingredient lists was recorded.

Cooking effect was measured as percentage of gain or loss of weight of the fish balls after immersion in boiling water for 4 min. The colour of fish balls was measured for lightness (L), redness (a) and yellowness (b) using colourmeter (Minolta CR 300, Japan). Five fish ball samples from each packet were used for colour measurement by cutting to a thickness of 4 mm (Yu, 1994). Moisture, protein, fat and ash contents of the samples were determined using the method described by AOAC (1990). Carbohydrate content was calculated by the difference.

Table 1. Ingredient used and price for Malaysian fish balls

Sample	Ingredients	Price (US$/kg)
A	Fish, wheat flour, potato flour, baking powder, MSG	2.18
B	Fish, modified corn starch, vegetable oil, ISP, salt, sugar, sodium pyrophosphate, MSG, permitted flavour enhancers	3.47
C	Fish, corn starch, salt, MSG	1.97
D	Surimi (Fish) wheat flour, salt, sugar, MSG, vegetable oil	2.37
E	Fish, salt, sago flour, wheat flour, ajinomoto	3.21
F	Fish, salt, sago flour, wheat flour, ajinomoto	3.21
G	Fish, wolf-herring fish, tapioca starch, sugar, salt	3.62
H	Fish, tapioca starch, sugar, salt	3.29
I	Not available	2.89
J	Fish, corn flour, salt	1.97

Folding test was carried out according to Lanier (1992). The fish ball samples were cut into 3 mm thick round slice at the middle of the ball and evaluated by a five stage method, as follows:

Grade	Condition
D	Breaks by finger pressure.
C	Cracks immediately when folded in half.
B	Cracks gradually when folded in half.
A	No crack after folding in half.
AA	No crack after folding twice.

A completely random design was used for the study. Data were analyzed using General Linear Model procedures of Statistical Analysis Systems (SAS) version 6.11 (SAS, 1994). Treatment showing significant differences ($p<05$) were subjected to Duncan's New Multiple Range Test.

Results and Discussion

Most of the fish ball samples were packaged and labelled with the ingredients used in the formulation. The ingredients used were different for different brand names. The type and species of fish used was not mentioned. The price of fish balls varied between US$ 1.97 and 3.47/kg, depending on the brand. In 1991, the price of fish balls in Thailand was US$ 1.18-3.54 and in Brunei it was US$ 3.72 (Chng *et al.*, 1991). The ingredients used and price for Malaysian fish balls are shown in Table 1.

The individual weight of each fish ball sample was significantly different ($p<0.05$). (Table 2) The individual weight varied between 6.25 and 15.14 g. However, the average weight of fish ball was about 10 g. There was a significant difference ($p<0.05$) in cooking effects on the weight of fish balls (Table 2). The weight of five fish ball samples increased from 0.83 to 8.21% after the cooking period while in the other samples, the weight decreased by 1.74-11.06%. The wide range of weight gain or loss may be correlated to the freshness of the fish balls, moisture content and other ingredients. The gain in weight after cooking was shown by the samples which was marketed at chilled/frozen temperature while the loss in weight were shown by samples which were marketed at room temperature (traditional markets). The different results may also be correlated to the amount of starch used in formulation. Higher content of starch resulted in an increase in the capacity to retain water since the starch molecules are attached to the myofibrillar protein after heating process (Yamprayoon *et al.*, 1991). The high moisture content and positive cooking effect in the case of sample D may be due to the water retaining capacity of the added surimi.

Since good quality fish balls are also represented by its whiteness, the colour of fish balls is an important factor which influences the consumers' preference. Results showed that the color of Malaysian commercial fish balls of different brands was significantly different ($p<0.05$) (Table 2). The L value varied between 62.14 and 76.85. The different L values may be correlated to the washing treatment used on the fish meat and

Table 2. Weight, cooking effects and colour values of Malaysian fish balls

Sample	Weight (g)	Cooking effects (%)	Lightness (L)	Redness (a)	Yellowness(b)
A	8.54±0.35^{f}	-5.70±4.20^{d}	66.71±0.99^{h}	-0.88±0.11^{a}	10.42±1.48^{a}
B	15.14±0.88^{a}	8.21±4.84^{a}	74.13±0.61^{b}	-2.15±0.07^{e}	5.96±0.35^{d}
C	9.27±0.67^{ed}	-3.46±2.41^{c}	76.85±0.57^{a}	-2.29±0.14^{f}	5.15±0.88^{d}
D	9.34±0.43^{ed}	1.30±0.95^{b}	72.99±0.51^{c}	-1.84±0.06^{d}	7.74±0.79^{c}
E	9.95±0.58^{bc}	1.62±1.59^{b}	67.44±0.49^{gh}	-1.20±0.11^{b}	9.40±0.78^{b}
F	10.42±0.62^{b}	0.83±0.53^{b}	67.70±0.35^{gf}	-1.55±0.21^{c}	9.21±0.35^{b}
G	9.88±0.71^{bc}	-7.12±2.69^{d}	71.57±0.79^{d}	-2.54±0.16^{g}	5.06±0.92^{d}
H	8.84±0.20^{ef}	-1.74±0.76^{e}	69.73±0.26^{e}	-2.37±0.07^{f}	5.20±0.57^{d}
I	9.80±0.57^{cd}	-11.06±1.22^{e}	68.35±1.09^{f}	-3.35±0.27^{h}	2.69±0.32^{e}
J	6.25±0.41^{g}	2.37±1.75^{b}	62.14±0.89^{i}	-0.75±0.19^{a}	10.73±1.94^{a}

* Value is the mean of five samples. Means in the same column with different superscripts are significantly different ($p<0.05$)

the starch used in the formulation. Yu (1994) reported that the colour of fish balls made from fish meat washed twice or thrice was significantly whiter than that made from unwashed and once washed fish meat. Starch used also influenced the L value. Yu & Yeang (1993) found that balls which were made from tapioca starch, showed higher L value followed by formulations using waxy maize, potato and corn starch. The redness 'a' and yellowness 'b' values of each fish ball sample were also significantly different ($p<0.05$) and varied between -3.35 and -0.75 and 2.69 and 10.73, respectively. The 'a' and 'b' values tend to decrease with increasing L value. Yu (1994) reported that the fish balls with higher L value tend to have lower 'a' and 'b' values.

The proximate composition of different brands of fish balls was significantly different ($p<0.05$) among all samples analyzed (Table 3). In Thailand, moisture and protein content of threadfin bream fish balls was about 80.13-80.58% and 11.09-11.25% respectively (Yamprayoon *et al.*, 1991). The shark fish balls from Indonesia showed different proximate composition. The moisture, protein and fat varied between 60 to 73%, 17 to 19%, 0.6 to 0.9%, respectively (Fawzya, 1992).

Table 3. Proximate composition of Malaysian fish balls

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
A	82.50±0.33^{e}	14.57±0.29^{a}	0.21±0.05^{de}	1.17±0.06^{f}	1.54±0.24^{g}
B	72.50±0.64^{1}	14.15±0.36^{b}	0.87±0.13^{b}	2.37±0.04^{bc}	10.10±0.34^{b}
C	83.38±0.16^{d}	11.14±0.17^{d}	0.11±0.06^{ef}	1.13±0.09^{f}	4.24±0.15^{e}
D	84.32±0.21^{e}	9.88±0.37^{g}	0.05±0.02^{f}	2.29±0.05^{e}	3.45±0.28^{f}
E	81.50±0.26^{f}	8.92±0.18^{1}	0.15±0.04^{def}	2.65±0.11^{a}	6.76±0.32^{d}
F	74.45±0.26^{h}	11.88±0.37^{c}	0.38±0.09^{c}	1.58±0.16^{e}	11.71±0.42^{a}
G	86.41±0.40^{b}	10.26±0.16^{f}	0.89±0.07^{bh}	2.10±0.19^{d}	0.34±0.16^{h}
H	83.41±0.12^{d}	10.61±0.14^{e}	1.95±0.31^{a}	2.46±0.04^{b}	1.58±0.33^{g}
I	89.92±0.17^{a}	8.30±0.17^{i}	0.08±0.03^{ef}	1.53±0.07^{e}	0.17±0.03^{h}
J	80.54±0.26^{g}	9.55±0.15^{h}	0.29±0.05^{cd}	2.25±0.04^{c}	7.36±0.14^{c}

* Value is the mean of triplicate analysis. Means in the same column with different superscripts are significantly different ($p<0.05$)

The varying proximate compositions of fish balls are mainly due to the different formulations used for fish balls production. Fish species and types of starch used also influenced the proximate composition. To minimize the varying protein content, the Malaysian Government has proposed that the minimum amount of fish meat to be used in fish balls formulation be not less than 50% (Laws of Malaysia, 1996).

Carbohydrate content of fish balls varied with the amount of starches used. In the past, starch was used in fish balls as a source of carbohydrate and to thicken the texture, but now they are used extensively as stabilizers, texturizers, water or fat binders and emulsifiers. Carbohydrate or starch also increases gel strength and freeze-thaw stability of the products if appropriately modified starches are added at proper level (Luallon, 1985). Generally, about 0 - 10% of starch is used for fish balls formulation (Yamprayoon *et al.*, 1991; Fawzya, 1992; Yu & Yeang, 1993).

Folding test is one of the simple methods used to measure the quality of fish balls. All the fish ball samples showed AA grade of folding test. Yu (1994) showed that AA grade of folding tests could be obtained by using washed minced fish in fish balls formulation. Unwashed minced fish will result in lower grade of folding test score. The AA grade of folding test indicated that the fish species, source of starch, storage method and ingredients used for fish balls formulation were acceptable for consumer preference on texture attribute.

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Effect of Washing on Polyphenoloxidase Activity in *Metapenaeus monoceros*

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The enzyme, polyphenoloxidase, is responsible for the black spot formation in prawn. Sulphiting agents such as sodium metabisulphite and sodium bisulphite are widely used in prawn processing factories to control black spot, but sensitive persons are allergic to these chemicals. Repeated washing with optimum volume of potable water helped to leach out polyphenoloxidase from prawn considerably. 92% of the enzyme was leached out in the first two washing in the case of headless shell-on prawn and 53% was leached out in case of shell on whole prawn.

Key words: Melanosis, polyphenoloxidase, *Metapenaeus monoceros*

The formation of black spot in prawn was studied in detail by many workers (Antony & Nair, 1968; Baily *et al.*, 1960; Kakimoto & Kanaswa, 1956). As soon as prawn is caught, it should be immediately kept in ice to maintain its quality. Exposure to tropical ambient temperature and air helps the formation of black spot. The initial black spots on the shell are not objectionable organoleptically and these spots can be removed by peeling. However, comparatively higher proportion of melanin formation affects the appearance with black spot stain on the meat. The enzyme, polyphenoloxidase, responsible for black spot formation, is present in the head portion of prawn and its activity in meat is minimum (Antony & Nair, 1975; Savagaon & Sreenivasan, 1978; Chakrabarti, 1993). Removal of the head initially, followed by washing of prawn, minimize melanin formation, but both the shell-on whole prawn and the shell-on headless prawn are major products of export. Various methods are commercially adopted to control melanosis e.g. (a) by reduction of the concentration of active enzyme, (b) by cutting off the source of oxygen supply by keeping the prawn under chilled water (Antony *et al.*, 1968), (c) by treating with some permitted reducing agents such as sodium bisulphite, sodium metabisulphite, ascorbic acid, etc. (Baily *et al.*, 1960; CSIRO, 1976; FAO, 1982) and (d) by combination of the above three methods. The sulphites are widely used by prawn processing factories. An allergic or sensitive person may develop asthma from the exposure to sulphur dioxide liberated from suhlphiting agents. King *et al.* (1991) reported the use of 4-hexylresorcinol and Taoukis *et al.*, (1990) reported the use of sulphydryl protease, ficin as alternatives to sulphiting agents in controlling melanosis in shrimps. The alternate chemicals are yet to be accepted and tested by prawn processing factories; but effect of residual chemical to human body is yet to be fully understood. Removal of the enzyme from the tissue is another effective way of controlling melanosis. Therefore an investigation was carried out to study the effectiveness of simple washing in the reduction of polyphenoloxidase activity in prawn.

Materials and Methods

Metapenaeus monoceros was collected from the landing place and brought to the laboratory under ice. Weighed amount of

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head portion of prawn before and after washing with water, was taken in a mortar and ground with purified sea sand in ice cold condition and extracted with two parts its weight of cold 10 mM sodium phosphate buffer (pH 7.0) containing 0.5% Triton and 0.5% polyvinyl pyrrolidone (pH 4.0). The extract was strained through cloth. The homogenate obtained was subjected to cold centrifugation at 10000 x g for 15 min. The supernatant was used as the crude extract of polyphenoloxidase (Kakimoto & Kanazawa, 1956). The enzyme was analysed by the modified Horowitz method (Savagaon & Sreenivasan, 1978). 3 ml of assay mixture, containing 1.5 ml of 100 mM sodium phosphate buffer, pH (6.5), 1 ml of 30 mM DL-3,4 dihydroxy phenyl alanine and 0.2 ml of enzyme preparation, was incubated at 28°C for 5 min. The formation of dopachrome was measured at 470 nm using a zero blank. The change in optical density in 5 min was expressed as the enzyme activity. The specific activity was calculated as the change in optical density per mg of protein. The protein concentration in the assay mixture was measured by the method of Lowry *et al.* (1951). The enzyme activity was also determined in the presence of inhibitors, such as L-ascorbic acid, L-cystein and sodium metabisulphite at different concentrations.

Shell-on whole prawns were washed in potable water in the ratio 1:0.5, 1:1, 1:2, 1:3, 1:5 and 1:7 separately by applying whirling motion for 10 min. After each wash, the wash-water was collected by draining. The polyphenoloxidase activity was estimated in the drained water.

Shell-on whole prawn was washed in potable water in the ratio 1:1 by applying whirling motion for 5, 10, 20 and 30 min separately. In each case, the wash-water was drained out, collected and used as enzyme source for the determination of polyphenoloxidase activity. Headless shell-on prawn and potable water in the ratio 2:1 were taken in a container and whirling motion was applied for 10 min for thorough washing. The wash-water was then collected by draining. The process was repeated consecutively four times. The drained water was collected separately and used for determination of polyphenoloxidase activity.

Results and Discussion

Table 1 showed that the polyphenoloxidase activity in the washed fraction was highest when the ratio between shell-on whole prawn and water was maintained at 1:2. Table 2 indicated that specific activity of polyphenoloxidase in water was highest after washing for 30 min. Table 3 showed that specific activity of polyphenoloxidase was about 30% lower in the extract of head portion of washed whole prawn, after washing (1:1) for 10 min. It was observed that during repeated washing the polyphenoloxidase activity in the water remained almost the same at the sixth and seventh washing (Table 4). Of the total released polyphenoloxidase activity, 37%

Table 1. Polyphenoloxidase activity in the drained fractions obtained by washing shell-on whole *M.monoceros* with different proportions of water

Prawn: Water ratio	Activity/ml (O.D.)	Amount of protein/ml (mg)	Specific activity
1:0.5	0.89	4.54	0.196
1:1	0.59	3.52	0.167
1:2	0.38	1.36	0.284
1:3	0.35	1.40	0.250
1:5	0.25	1.02	0.240
1:7	0.13	0.68	0.191

Table 2. Effect of time of exposure on the release of polyphenoloxidase from shell-on whole *M. monoceros* during washing with water

Time given for washing (minutes)	Activity/ml (O.D.)	Protein/ml (mg)	Specific activity (O.D./mg protein)
5	0.12	1.20	0.100
10	0.9	5.33	0.168
20	1.0	6.83	0.148
30	2.6	9.66	0.260

Table 3. Polyphenoloxidase activity in the extracts of head portions of *M. monoceros*

Head portion	Activity*/ ml (O.D.)	Protein/ ml (mg)	Specific activity**
Unwashed whole prawn	2.6	13.25	0.196
Washed whole prawn	1.8	13.25	0.136

* The activity is expressed as the change in optical density (O.D.) per ml of enzyme in 5 minutes at 20°C.

** The specific activity is expressed as the change in O.D. per mg of protein in 5 minutes.

Table 4. Release of polyphenoloxidase from shell-on *M. monoceros* during successive washing.

Successive stages of washing	Activity/ ml (O.D.)	Protein/ml (mg)	Specific activity	% enzyme released in washing
Whole shell-on prawn				
1st washing	1.000	3.66	0.27	37.0
2nd washing	0.425	1.66	0.25	15.7
3rd washing	0.325	1.64	0.19	12.0
4th washing	0.300	1.33	0.22	11.1
5th washing	0.275	1.25	0.22	10.1
6th washing	0.255	1.25	0.13	8.3
7th washing	0.150	1.25	0.12	5.5
Headless shell-on prawn				
1st washing	0.800	5.0	0.16	66.6
2nd washing	0.300	2.5	0.12	25.0
3rd washing	0.125	1.2	0.10	8.3
4th washing	0.100	1.2	0.08	3.3

Table 5. Effect of different inhibitiors upon polyphenol odidase isolated from *M. monoceros*

Inhibitors	Concentration of the inhibitor (mM)	% of inhibition
Ascorbic acid	0.025	36.6
	0.05	70.0
	0.1	91.1
L. cystein	0.025	37.1
	0.05	53.7
	0.1	88.5
Sodium metabisulphite	1	22.2
	2	50.0
	4	72.7

came in the first washing followed by 16% in the second washing. Table 4 also shows that about 67% of the total released polyphenoloxidase activity appeared in the first washing of shell-on headless prawn, and 25% in the second washing.

It was also observed that 0.05 mM ascorbic acid or L-cystein was sufficient to bring about 50% inhibition of polyphenoloxidase (Table 5), while the requirement for sodium metabisulphite was at least 2 mM to have the same effect.

The result showed that 92% of the enzyme leached out in the first two washings in case of headless shell-on prawn, but it was 53% in case of shell-on whole prawn. Washing shell-on whole and headless prawn twice with water in the ratio 1:2 for 10 min removed the polyphenoloxidase content considerably. Residual enzymatic activity in washed prawn can be controlled by maintaining cold chain system up to consumer's end. As a precautionary measure, the dipping of the washed prawn in the solution of permitted chemicals will effectively prevent black spot/melanosis.

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Changes in Biochemical Composition in Indian Major Carps in Relation to size

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Total biochemical composition of freshwater major carps, *Labeo rohita* -(rohu); *Catla catla* (catla); *Cirrhinus mrigala* (mrigal), in relation to size was evaluated. The water soluble nitrogen fraction constituted about 21% of the total nitrogen. Salt soluble fraction constituted 55 - 60% of the total nitrogen. Non-protein nitrogen represented 12% of the total nitrogen in all the cases except mrigal of small size (9.36%). The insoluble connective tissue contributed to 2-3 per cent of total nitrogen. The monounsaturated fatty acids (MUFA) formed 31-39% of the total fatty acids and 60-68% of the MUFA in freshwater carps was C18:1. The essential amino acids contributed to 41 to 51% of the total amino acids in freshwater major carps. Aromatic amino acid content was slightly higher compared to marine fishes while the proportion of proline was less. Variations in composition in relation to size are discussed. The autolytic activity was significantly higher in small fish in all the three species

Key words: Major carps, amino acids, fatty acids, autolysis, rohu, cutala, mrigal

The composition of the fish muscle varies according to many factors such as sex, size, stages of maturity and season. Starvation resulted in a decrease in protein and lipid contents coupled with an increase in water content (Wendakoon & Shimizu, 1991). The proximate composition of a number of marine, freshwater and brackish water fish has been reported (Gopakumar, 1997; Mukundan *et. al.*, 1986). Understanding the functional properties is of utmost importance for utilizing the fish in the preparation of value added products and the functional properties depend on the composition of the meat. Earlier reports indicate that changes in composition may occur as a result of gonadal maturity (Itoh *et al*, 1995; Montecchia *et al*, 1997). The properties of actomyosin are particularly affected during maturation. Pre-spawning fish was found to contain lower actomyosin content (Montecchia *et al.*, 1997). However, information on the changes in composition with size of the fish is relatively scanty. Hence, an attempt has been made to study the biochemical composition of freshwater major carps - rohu, catla and mrigal - in relation to size.

Materials and Methods

Fish (rohu - *Labeo rohita*, catla - *Catla catla* and mrigal - *Cirrhinus mrigala*) were collected from the culture ponds in absolutely fresh condition and brought to the laboratory partially iced. Fish of two different sizes - smaller size weighing around 500 g and a commercial size weighing above 1000 g were selected based on the gonadal maturation stage of the fish.

The fish were washed thoroughly to remove slime, dirt etc. and skin-less bone-free fillets were made. The fillets were homogenized by passing through a hand extruder and mixed thoroughly. The temperature of the fish and the mince were maintained below 5°C throughout.

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Moisture, protein, fat and ash were determined according to the methods of AOAC (1990). The muscle lipids were extracted by using 2:1 mixture of chloroform and methanol (Folch *et al.*, 1957). The quantitative derivatisation of fatty acids to fatty acid methyl esters (FAME) was carried out using boron triflouride-methanol (BF3-CH3OH) reagent (Matcalfe *et al.*, 1966). The FAMEs were analysed by a Chrompack CP 9001 gas chromatograph equipped with a flame ionisation detector.

The samples for amino acid analyses were prepared according to the method of Ishida *et al.*, (1981). The sample thus prepared was filtered using a membrane filter of 0.45µm and 20µl was injected into Shimadzu HPLC - LC 10 AS, fitted with a packed column (ISC-07/S1504-Na). The amino acid identification was done by non-switching flow method and fluorescence method after the post column derivatisation of amino acids with o-phthalaldehyde. Tryptophan was estimated as per the method of Sastry & Tummuru (1985) after alkali hydrolysis of the sample using 5% sodium hydroxide at 110°C for 24 hours.

The soluble proteins were characterized by the method of Hashimoto *et al* (1979) with some modifications. In place of phosphate buffer, bicarbonate and sodium chloride-bicarbonate buffer of appropriate ionic strengths were used. The autolytic activity of the muscle was studied as described by Green & Babbit (1990) and the Folin positive material in the supernatant was determined by the method of Herriot, (1955). The activity was expressed as µmol tyrosine released per ml test sample per minute.

Results and Discussion

Average moisture content (Table 1) noticed for fresh rohu, catla and mrigal flesh was in the range of 77 -81%. The higher moisture content of the fish flesh noticed was due to the partial icing of the fish for 24 hours prior to processing. Moisture content in the range of 76 to 77% has been reported for the same fish (Mukundan *et al*, 1986; Joseph *et al.*, 1990; Gopakumar, 1997). Larger sized fish had marginally higher moisture levels, 2.8% in rohu, 3.1% in mrigal and only 1.9% in cutla.a

Protein content varied from 16-19% in the samples analysed with the lowest value of 16.13% for rohu - big, and the highest value of 19.25% for mrigal - small. These values were in agreement with those reported earlier (Gopakumar, 1997). In rohu and mrigal, the proportion of protein was higher in the smaller fish. The difference was more than 10% in these cases. In catla, there was only marginal difference between the two groups.

The total protein is the sum of different protein fractions - water-soluble sarcoplasmic

Table 1. Proximate composition* of rohu, catla and mrigal

	Rohu		Catla		Mrigal	
	Small	Big	Small	Big	Small	Big
Weight (g) / length (cm)	560 / 39	1200 / 57	560 / 36	1350 / 45	560 / 38	1600 / 56
Weight of gonad, (g)	2.25	4.07	2.21	3.98	2.78	4.35
Moisture %	79.26	81.75	80.72	79.23	77.82	80.00
Crude protein, % (N x 6.25)	18.41	16.13	16.98	16.54	19.25	16.29
Fat, %	1.01(4.99)	2.43 (13.32)	1.12 (5.81)	2.09 (10.84	1.25 (5.64)	2.13 (10.65)
Carbohydrate, %	0.85 (4.10)	1.82 (8.78)	1.12 (5.81)	1.89 (9.80)	1.12 (5.05)	1.95 (9.75)
Ash, %	1.02 (4.92)	1.15 (5.54)	1.17 (6.09)	1.32 (6.85)	1.56 (7.03)	1.97 (9.85)
Calorie	86.13	93.67	82.48	92.53	92.73	92.13

* Values are average of triplicate analysis
Value in parenthesis denote dry weight basis

proteins including the non-protein nitrogen, the salt soluble myofibrillar protein and the stroma proteins and these individual proteins determine the texture, and other functional properties. The water-soluble nitrogen fraction (Table 2) constituted about 18 -25% of total nitrogen. The values were in the lower range in rohu - big and catla - small. In these two cases the alkali soluble denatured protein content showed an increase, suggesting the possible denaturation of water-soluble proteins during the handling process. These results are comparable to other reports in these fish (Joseph *et al.*, 1990). No correlation could be established in relation to size.

The salt soluble fraction (Table 2) reflect the actual content of structural proteins, i.e the myofibrillar proteins which are of significance as far as the production of surimi and similar products are concerned. The salt soluble fraction (SSN) constituted 55 - 60% of total nitrogen except in catla-small and mrigal-big where the contents were marginally lower. This could be due to the insolubilization of this fraction as a result of the handling condition and is explained by the increased content of alkali soluble nitrogen.

The non-protein nitrogen constituted about 13.14% (Table 2) of the total nitrogen in all the cases excepting mrigal small (9.36%). Not much difference was noticed between small and big fish in the case of rohu and catla but more than 30% increase was noticed in big mrigal compared to the small fish. Generally the non-protein nitrogen constitutes about 9 - 18 per cent in teleosts and 33 - 38 per cent in elasmobranchs (Haard *et al.*, 1994). These water-soluble fractions are of significance to food technologists because of their association with the taste of the seafood and contribution to spoilage. Joseph *et. al.*, (1990) reported similar results in the same fish during ice storage studies. The insoluble connective tissue contributed to 2-3 per cent of total nitrogen, which is well within the range of 2-5 per cent reported for bony fish (Sikorski & Borderas, 1994).

Fat content of rohu, catla and mrigal were in the range 1.0- 2.5%. A clear distinction in fat content between small and big fish was seen after calculating on dry weight basis. About three-fold increase was noticed in big fish in the case of rohu while in catla and mrigal the increase was about two fold.

Ash content of the fish showed only a marginal variation between species (Table 1). Variations in ash content with size of the fish also did not show any clear trend. The large sized mrigal had higher ash content, but in the case of the other two species, the differences were insignificant.

Though the carbohydrate content is almost negligible in fish, the content in freshwater fish was slightly higher. The carbohydrate content almost doubled in all the three fish with size (Table 1). The calorific value of the fish increased with size and may be related to the increase in fat content with size.

Table 2. Different nitrogenous fractions* in rohu, catla and mrigal

	Rohu		Catla		Mrigal	
	Small	Big	Small	Big	Small	Big
Water soluble nitrogen	21.69	18.61	19.81	25.77	21.42	21.16
Salt soluble nitrogen	58.31	55.43	50.94	54.64	54.36	51.54
Non protein nitrogen	12.88	12.79	13.84	12.72	9.36	14.33
Alkali soluble nitrogen	1.69	10.08	10.89	6.56	8.35	8.92
Stroma nitrogen	2.37	3.49	2.63	2.09	2.82	2.68

* Per cent of total nitrogen
Values are average of triplicate analysis

Table 3. Fatty acid composition* of fish meat from rohu, catla and mrigal (area %)

Fatty acid	Rohu		Catla		Mrigal	
	Small	Big	Small	Big	Small	Big
C12	0.52	0.26	0.00	0.25	0.83	0.26
C13	0.15	0.00	0.40	0.26	0.26	0.00
C14	5.12	2.86	2.20	3.05	4.39	2.86
C14:1	0.59	1.45	0.77	1.91	0.97	2.86
C15	2.75	1.34	1.63	1.21	2.39	1.34
C16	21.58	25.08	23.10	25.65	19.43	27.28
C16:1	9.64	8.36	8.95	9.12	9.24	8.28
C17	1.84	3.66	2.25	4.05	2.10	0.96
C18	3.65	0.44	0.21	0:31	4.21	0.44
C18:1	22.35	24.88	26.36	26.17	19.54	25.56
C18:2	6.25	5.77	5.00	6.12	7.34	4.99
C18:3	2.56	2.45	3.10	2.25	2.35	1.90
C18:4	1.95	1.79	2.04	1.95	1.42	1.36
C19	0.00	0.30	0.48	0.52	0.00	0.30
C20:1	0.18	0.01	0.25	0.18	0.23	0.00
C20:2	0.84	0.79	0.80	0.94	0.75	0.89
C20:4	5.28	4.32	4.88	4.57	6.23	4.58
C20:5	2.64	1.41	2.80	2.75	2.56	1.50
C22:1	0.62	0.49	0.92	0.88	0.51	0.56
C22:6	8.12	7.96	6.00	6.12	7.46	7.86
C24:1	1.51	1.20	1.95	1.45	1.48	1.20
Total SFA	35.61	33.94	30.27	35.3	33.61	33.44
Total MUFA	34.89	36.39	39.20	39.71	31.97	38.46
Total PUFA	27.64	24.49	24.62	24.70	28.11	23.08

* Values are average of triplicate analysis

The fatty acid composition was slightly different from that of marine fish (Table 3). Generally, saturated fatty acids (SFA) form 15 - 35% of total fatty acids in marine fish (Ackman, 1989). SFA constituted 30 - 36 % in the three fish with the highest proportion in rohu-small and the lowest in catla-small. This included about 2-5% of odd numbered SFA also. Among the SFA, palmitic acid (16:0) content was 20-25% of the total fatty acids and is comparable to that in marine fish (Nair, 1998). Palmitic acid accounts for upto 60% of SFA in marine fish (Ackman & Eaten, 1966) and in the freshwater major carps analysed in the study, 16:0 constituted 60-80% of the total SFA, the exception being mrigal-small where it was just 58%.

The monounsaturated fatty acids (MUFA) formed 31-39% of the total fatty acids in the three major carps and its content was higher than that reported for marine fish (Nair & Gopakumar, 1978). 60-68% of the MUFA in freshwater carps was C18: 1 (oleic acid). Fish from Indian waters were reported to contain about 13% of C18: 1 (Nair, 1998), against the more than 20% seen in freshwater major carps studied.

The polyunsaturated fatty acid (PUFA) accounted for 23 - 28% of the total fatty acids among the different samples, with the maximum content in mrigal-small and minimum in mrigal-big. 68-71% of the PUFA was constituted by C18: 2 (n-6), 20: 4 (n-6) and C22: 6(n-3) with C22: 6 predominating in all the cases. The C22: 6 content, however, was comparatively less than that of marine fish. The PUFA content in the case of marine fish ranged from 28 to 57% with C20:5 and C22: 6 predominating and constituting about 50% in most cases (Nair & Gopakumar, 1978). The other principal fatty acid from marine source C20: 5 was present in comparatively lower proportions in freshwater fish. The C20: 4 (n-6) content was marginally higher but the C22: 6 was very much lower than that of marine fish.

In the case of rohu, the total SFA and PUFA content decreased with increase in size while the MUFA content increased marginally. Almost similar trend was noticed in the case of mrigal but the increase in MUFA was about 20%. In catla, the proportion of saturated fatty acids was higher in the larger fish. However, the available data do not indicate any definite relationship between fatty acid composition and the size of the fish.

Glutamic acid was the major amino acid accounting for 15 - 20% of total amino acids and this result is in accordance with the earlier report for rohu and mrigal (Mukundan *et.al.*, 1986). The glutamic acid content was marginally higher than that in marine fish (Gopakumar, 1997) and maximum content was in rohu - small and minimum in catla - big (Table 4). Aspartic acid (10-12%) occupied the second position. Marginal

Table 4. Amino acid composition of fish meat* from rohu, catla and mrigal (g/100g protein)

Amino acid	Rohu		Catla		Mrigal	
	Small	Big	Small	Big	Small	Big
Aspartic acid	10.99	11.29	10.26	11.21	11.59	12.25
Threonine	3.95	4.55	4.37	4.18	4.25	4.56
Serine	4.00	4.24	4.24	4.03	4.05	4.26
Glutamic acid	20.06	18.98	18.21	15.63	17.88	18.02
Proline	2.73	3.03	3.48	3.91	3.47	3.52
Glycine	4.99	4.95	3.31	3.19	5.00	5.02
Alanine	7.80	7.07	8.79	6.76	7.07	7.25
Cysteine	0.76	0.00	2.01	2.13	0.84	0.52
Valine	4.05	5.07	5.21	4.07	4.80	4.92
Methionine	1.79	2.18	2.25	1.86	1.91	2.14
Isoleucine	3.94	4.56	4.91	4.33	3.68	2.58
Leucine	8.58	8.89	9.33	8.45	7.09	7.98
Tyrosine	3.56	3.48	3.82	3.27	3.51	3.66
Phenylalanine	3.41	4.49	4.54	3.92	4.20	4.32
Histidine	3.78	5.09	3.95	3.83	4.97	5.28
Lysine	3.01	5.40	7.40	8.20	5.34	3.21
Tryptophan	1.12	1.49	1.27	1.18	1.39	1.45
Arginine	5.58	6.24	7.89	12.07	5.58	5.78

* Values are average of triplicate analysis

decrease in glutamic acid and a similar increase in aspartic acid were noticed with growth.

The essential amino acids contributed to 41 to 51% of the total amino acids. Catla showed a marginally higher content of essential amino acids. Similarly, the content of histidine, an amino acid of nutritional significance in children, was higher in mrigal. The lysine content in these species was lower than that in marine fish. Aromatic amino acids formed 8 to 10% of the total amino acids, which was slightly higher than that of marine fish. The increase is related to the higher tyrosine content associated with these fish. Proline content of freshwater carps was lower than that of marine species indicating the lesser connective tissue content of these fishes. However, there are no marked differences in the amino acid pattern between the small and big fish in all the three species.

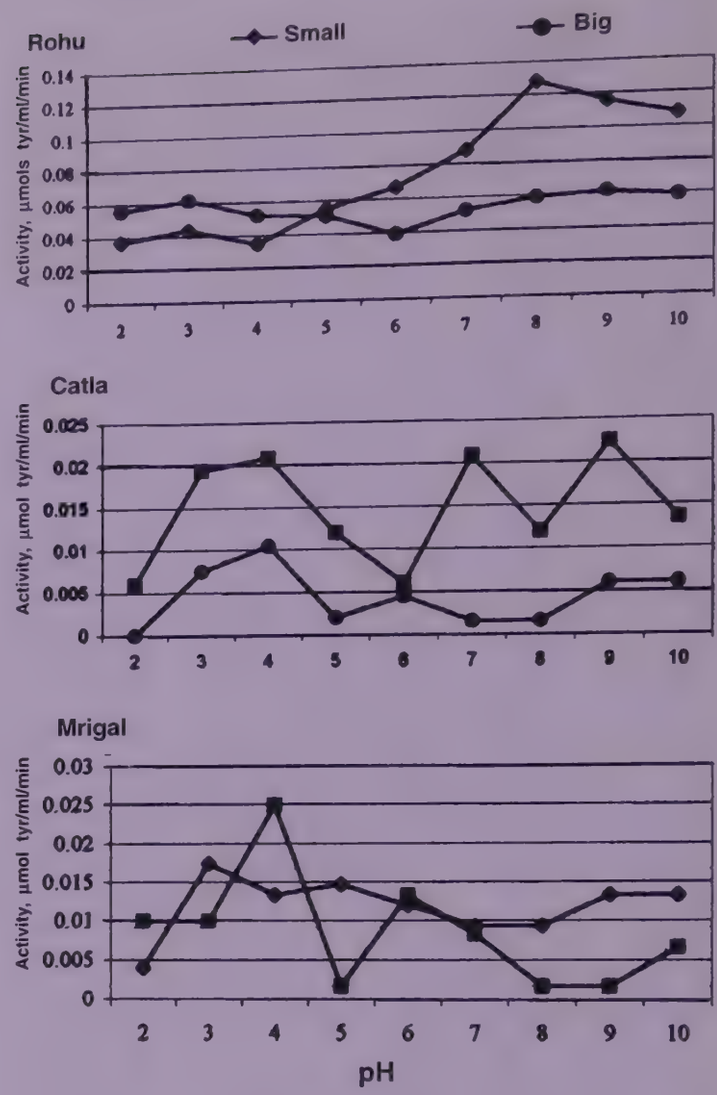

Fig. 1. Autolytic activities in small and big size rohu, catla and mrigal at different pH

The autolytic activity was significantly higher in small fish in all the three species studied (Fig 1). Among the smaller fish, catla showed several times higher activity at alkaline, neutral and acid pH ranges. Mrigal followed, with activities at almost all pH ranges while rohu showed lowest activity among the three fish, with maximum activity in the alkaline range. Rohu-small showed low activity at lower pH ranges (pH 2-5) but rohu - big showed marginally higher activity. However, above pH 5, there was an increase in autolysis and at pH 8 the activity was more than double the activity at pH 5. Above pH 8, the activity decreased marginally. Catla showed high activity at pH ranges 3, 4, 7, 9 and 10, moderate activity at pH 5 and 8 and low activity at pH 6. In big fish, higher activity was noticed in the acid and neutral pH ranges.

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Survival and Growth of *Listeria monocytogenes* in Refrigerated Fish

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The ability of *Listeria monocytogenes* to survive and grow in fish at refrigerated condition was studied. Fresh, white sardine *(Kowala coval)* were washed and inoculated with *L. monocytogenes* (NCTC 1994) at a level of 104 cfu.g^{-1}. The inoculated fish were kept at room temperature (28±2°C) for 6 h and then stored at refrigerated condition (4±1°C). The fish was found to have a load of 10^5 cfu.g^{-1} of *L. monocytogenes* after the temperature abuse. Though there was a minimal increase in the count during the first two days of refrigerated storage, a slight reduction in counts was observed on the 4th day of chilled storage and thereafter the counts did not show appreciable change. The results suggest that chilling does not have a significant effect on the control of *L. monocytogenes* in fish and there is a possibility of contaminated seafood acting as a vehicle of infection.

Key words: ***Listeria monocytogenes*, refrigeration, survival, growth, white sardine, *Kowala coval***

Listeria monocytogenes has been recognized as an important food-borne pathogen since 1981 and seafoods have been implicated in several sporadic cases of listeriosis (Facinelli *et al.*, 1989; Frediksen, 1991; Baker *et al.*, 1993) and in two outbreaks (Lennon *et al.*, 1984; Reido *et al.*, 1990). Buchanan *et al.* (1989) isolated *L. monocytogenes* from 15% of uncooked seafood samples. *L. monocytogenes* was found to be present in 24% of raw fish samples (Hartemink & Georgsson, 1991). Walker *et al.* (1990) reported the growth of *L. monocytogenes* at refrigerated temperatures. Harrison *et al.* (1991) determined the fate of *L. monocytogenes* in packaged and iced fish. Against this background, the effect of chilling on the survival and growth of *L. monocytogenes* in fish stored under refrigerated condition was studied.

Material and Methods

Fresh white sardine *(Kowala coval)* were procured from Mangalore fish market, washed and kept in ice till they were used for the inoculation studies. They were initially tested for the presence of listeriae. The standard *L. monocytogenes* (NCTC 1994) bath was prepared by growing the culture overnight at 37°C in 300 ml of trpticase soy broth (TSB) containing 0.6% yeast extract. Then, the culture was centrifuged at 5000 rpm for 10 min at 4°C to remove the cell pellet. The cell pellet was washed twice in sterile physiological saline and suspended in 5 ml saline and the whole cell suspension was added to 1 l sterile saline and the load of listeriae was tested immediately after the addition of *L. monocytogenes* by plating serial dilutions on to modified McBrides listeria (MML) agar plates and incubating at 37°C for 24-48 h. Typical colonies were confirmed by Gram reaction as well as catalase and motility tests (Jones & Seeliger, 1992).

The fish were immersed in the inoculation bath for 1 min and then drained. The inoculated fish were kept at room temperature (28±2°C) for 6 h, so as to simulate the fish handling conditions practised by the local fishermen, and then, stored at refrigerated temperature (4±1°C). The load of *Listeria* in the treated fish was determined immediately after the dip treatment and after 6 h at room temperature. The survival and

growth of *L. monocytogenes* on the refrigerated fish was monitored at regular intervals until the fish was apparently spoiled.

Results and Discussion

The fish samples were initially free from listeriae. On experimental inoculation, they were found to have *L. monocytogenes* load of 2.64×10^4 cfu.g^{-1} (Table 1). After the temperature abuse, the fish was found to have a count of 9.84×10^5 cfu.g^{-1} of *Listeria* which was higher than the initial load. A marginal increase in counts of *Listeria* was noticed in fish during the first two days of refrigerated storage. Later, a slight reduction in the count was observed on the fourth day of storage and thereafter it remained almost at the same level, till the fish was totally spoiled.

The results showed that *L. monocytogenes* could survive and grow in fish under refrigerated condition. It also demonstrated that exposure of infested sample to ambient temperature even for 6 h could lead to a 10 fold increase in counts of *Listeria* (Table 1). On the second day of storage at refrigerated condition, one log increase in the count was noticed, but on the 4th day, one log reduction in the count was observed. Thereafter, no significant change in the counts was observed till the fish was totally spoiled. However, Lovett *et al.* (1990) found that there was a five log increase in the count when *L. monocytogenes* was artificially inoculated into white fish and surimi and stored at 7°C for 14 days. In present study, the storage time was only 8 days and the initial counts were also high. Nevertheless, there was a 1-2 log units increase in the counts during the storage.

Table 1. Survival of *Listeria monocytogenes* in chilled fish

Sample	Storage period	*L. monocytogenes* counts (cfu.g^{-1})
Fresh	-	Nil
Inoculated	0	2.64×10^4
Stored at room temperature	6 h	9.84×10^5
Stored at 7°C	1 day	7.96×10^5
	2 day	1.34×10^6
	3 day	1.66×10^6
	4 day	7.60×10^5
	5 day	2.38×10^5
	6 day	3.68×10^5
	7 day	6.16×10^5
	8 day	6.16×10^5

Farber (1991) also reported that *L. monocytogenes* grew fairly well on salmon, increasing about 2-3 logs within 7 days at 4°C. But, Wang & Shelef (1992) reported that *L. monocytogenes* load in refrigerated cod fish fillets remained unchanged during the first 10 days of storage at 5°C, and the numbers increased by more than one order of magnitude at the end of 17 days of storage. The results are in agreement with the findings of Dorsa *et al.* (1993) who reported that a temperature of 6°C would support the growth of *L. monocytogenes* and short term temperature abuse at 12°C would induce rapid growth of the organism. The results of the study suggest that low numbers of *L. monocytogenes* present in raw seafoods might multiply and reach dangerous levels during storage at refrigerated temperatures.

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Cellulolytic Bacteria in Marine Wood Borers

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Four wood borers *(Sphaeroma terebrans, S. annandalei, Martesia striata* and *Teredo furcifera)* were collected from four stations (Akathumuri, Edava Nadayara, Neendakara and Ashtamudi) of Kerala Coast. The cellulolytic bacterial population in *S. terebrans* during different seasons were investigated. Maximum population was recorded during monsoon season in the hind gut region. Samples from Ashtamudi and Akathumuri recorded the maximum cellulolytic bacteria in *S. terebrans*. Similarly a survey of the cellulolytic bacteria in different wood-borers collected from Ashtamudi revealed that *M. striata* harboured the maximum cellulolytic bacteria and *S. annandalei* showed the minimum population. Bacterial isolates randomly selected, exhibited maximum cellulolytic activity of 350µg glu.ml^{-1}h^{-1} in *Micrococcus* (AQB Th3) isolated from *S. terebrans* and minimum, 37µg glu.ml^{-1}h^{-1} in *Corynebacterium* (AQB Th2) isolated from *S. annandalei*. The enzyme activity in the mouth and viscera of wood borers was investigated. It was noted that maximum cellulolytic activity was in the mouth region though not low in the visceral region.

Key words: Wood borers, cellulolytic bacteria

Cellulose is distributed widely in submerged wood (John, 1964) and in seaweeds (Aspinall *et al.*, 1958). The cellulose material is decomposed by cellulose decomposing microbes in nature, (Dhevendaran *et al.* 1992; Rajasree, 1996). Certain marine *Vibrio* spp., *Streptomyces* and fungi exhibited cellulolytic activity (Balasubramaniam *et al.*, 1975; Venugopalan *et al.*, 1986), Florkin & Lozet (1949) have demonstrated the presence of cellulolytic bacteria, decomposing cellulose in the digestive tract of the snail *H. pomatia*. Polychaetes such as *Nereis virens* fed on algae possessed cellulase enzyme (Lewis & Whitney, 1968). Cutter & Rosenberg (1972) investigated cellulolytic bacteria in the digestive system of shipworm. Boyle & Mitchel (1980) observed the interaction between microorganisms and marine wood boring crustaceans. Waterbury *et al.* (1983) isolated nitrogen fixing cellulolytic bacteria from a specialized gland of the shipworm, known as the gland of Deshayes. Adhesive properties of symbiotic bacteria from a wood boring marine shipworm was investigated by Syed *et al.* (1990). Although a few reports are available at present on distribution of wood borers (Nair & Dharmaraj, 1979, 1980, 1982) on cellulolytic bacteria and cellulose synthesis in wood borers, information on the combined cellulolytic activity by marine wood borers and associated bacteria in Southwest of India is totally lacking. This work was aimed at investigating cellulolytic activity of bacterial strains associated with wood borers and the enzyme activity in the animals themselves to assess, whether any association existed between bacteria and the host, based on cellulose metabolism.

Materials and Methods

The present investigation was carried out over a period of one year (Jan. - Dec., 1992). The wooden planks containing wood borers were removed from the main trees in coastal regions of Akathumuri, Edava Nadayara, Neendakara and Ashtamudi and placed in polythene bags. They were brought to the laboratory in minimum time to avoid variations in microbial load. In the laboratory, the wood borers *(Sphaeroma annandalei, S. terebrans, Martesia striata* and *Teredo furcifera)* were removed from the wood using sterile needle and forceps into sterile

petridishes under aseptic conditions, unless otherwise stated. The animals were dissected into head, thorax and abdomen and also digestive tract. They were independently transferred into 99 ml of sterile 50% seawater blank and homogenized using a sterile tissue homogeniser. Standard plating technique was followed employing the method adopted by Dhevendaran (1977) and Dhevendaran & Maya (1998).

Randomly selected bacterial strains were identified to the generic level following the methods of Simidu & Aiso (1962) and Staley *et al.* (1989).

For assay of cellulolytic activity, 0.1 ml of 24 h old culture was inoculated into 8 ml nutrient broth, prepared in phosphate buffer at pH 7.0 with 1% of carboxy methyl cellulose (CMC) and incubated for 48 h at room temperature (28±2°C). After the incubation period was over, the bacterial biomass was estimated by measuring at 600 nm in UNICAM - spectrophotometer. To estimate cellulase activity, 2 ml of culture broth was mixed with 2 ml of copper reagent and heated in boiling water bath for 20 min. After sufficient cooling, 2 ml of arsenomolybdate was added and blue colour was developed. The whole content was centrifuged at 10,000 rpm for 10 min. The supernatent was diluted with 4 ml of distilled water and then it was read at 495 nm in the UNICAM-spectrophotometer (Dhevendaran *et al.*, 1992).

Results and Discussion

Seasonal variations of cellulolytic bacterial load on the exoskeleton of *S. terebrans* is given in Fig.1a. During post-monsoon season maximum bacterial population ($25 \times 10^4.g^{-1}$) was found in abdominal part and minimum ($1.82 \times 10^4.g^{-1}$) population was noticed during pre-monsoon season in the head region. This pattern was similar to that observed in termites by Jaisree *et al.* (1985). The cellulolytic bacterial load in the digestive tract of *S. terabrans* is presented in Fig.1b.

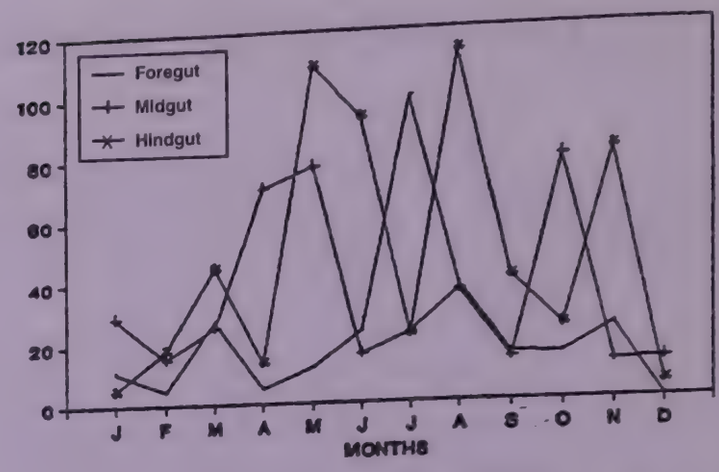

Fig. 1 a. Seasonal variations of cellulolytic bacteria isolated from the exoskeleton of wood-borer, *S. terebrans*

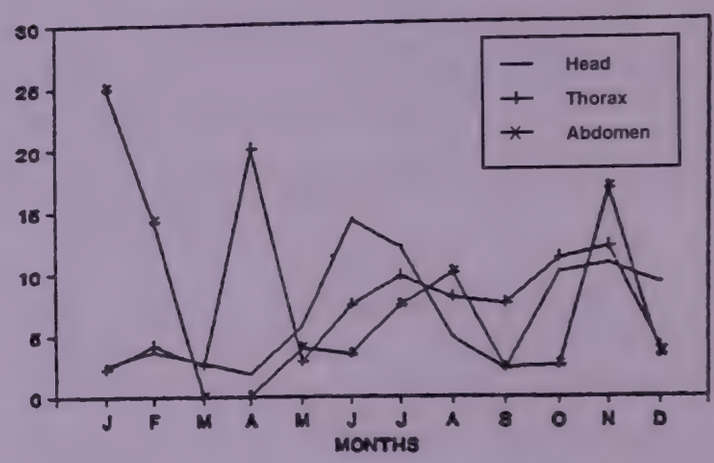

Fig. 1 b. Seasonal variations of cellulolytic bacteria isolated from the digestive tract of wood-borer, *S. terebrans*

Seasonal variations of the microflora in the digestive tract of wood borers revealed that hind gut harboured maximum cellulolytic bacteria ($112.9 \times 10^4.g^{-1}$) during monsoon months and minimum ($4.76 \times 10^4.g^{-1}$) during postmonsoon season. Kasinathan *et al* (1973) observed that three species of land snail harboured maximum cellulolytic bacteria in the post-stomach and intestine. Thayer (1976) reported the occurrence of maximum cellulolytic bacteria in the hind gut of termites. It was seen that there were no statistically significant differences in the bacterial load between seasons or body parts. The slight differences in the cellulolytic bacterial population may be due to changes in the external environmental factors like salinity and temperature, which in turn influence growth of cellulolytic bacteria. The proportion of cellulolytic bacteria varied

Table 1. Cellulolytic bacterial load on the exoskeleton and alimentary tract of *Sphaeroma terebrans* collected from Ashtamudi lake

Sources	THB x $10^6.g^{-1}$	Cellulolytic Bacteria x $10^6.g^{-1}$	% of cellulolytic bacteria
Head	62.82	27.00	42.98
Thorax	56.87	29.00	51.00
Abdomen	61.27	22.00	35.91
Fore gut	87.53	44.00	50.27
Mid gut	117.68	48.00	40.79
Hind gut	113.20	59.00	52.12

from about 36% of the total bacterial load in the abdomen to about 52% in the hind gut (Table 1).

It was observed that most of the gut microflora were cocci. In a similar study, Breznak, (1975) isolated cellulolytic cocci and coccobacilli from the gut of *Reticuliterms flavipes* and *Copotermes formosanus.*

Cellulolytic bacterial population in the different body parts of the exoskeleton of *S. terebrans* collected from different regions showed wide variations (Table 2). Maximum population was in the sample collected from Ashtamudi lake and minimum in the animal collected from Neendakara region.

Cellulolytic bacterial load in different parts of alimentary canal of selected wood borers from Ashtamudi is shown in Table 3. It was noted that the foregut of *M. striata* harboured the maximum number of cellulolytic bacteria and the hind gut of *S. annandalei,* the minimum. In the present study, the cellulose decomposing bacteria may contribute to the cellulase pool in the

Table 2. Cellulolytic bacterial population on the exoskeleton of *Sphaeroma terebrans* collected from different loctions

Sources	Cellulolytic bacterial population, x $10^6.g^{-1}$		
	Head	Thorax	Abdomen
Akathumuri	8.57	26.53	14.89
Edava Nadayar	18.00	25.66	13.75
Neendakara	4.00	7.55	2.00
Ashtamudi	25.00	20.00	4.00

Table 3. Cellulolytic bacterial population in the alimentary tract of different wood borers collected along the Ashtamudi lake

Sources	Cellulolytic bacterial population, x $10^6.g^{-1}$		
	Fore gut	Mid gut	Hind gut
S. terebrans	10.00	25.00	2.00
S. annandalei	10.00	15.75	5.62
M. Striata	30.00	20.19	15.25
T. furcifera	2.00	8.50	15.00

animal to a limited extent. There seems to exist a symbiotic association between the wood borers and the cellulolytic bacteria.

Cellulolytic activities of randomly selected 24 isolates from exoskeleton and alimentary tract of different wood borers are presented in Table 4. *Micrococcus* isolated from mid gut of *S. terebrans* showed maximum growth (1.40 OD) and elevated

Table 4. Cellulolytic activity in selected bacterial strains

Bacterial culture	Sources	Isolate number	Growth (OD at 6.0 nm)	Activity (µg glu. $ml^{-1}.h^{-1}$
Arthrobacter	*S. terebrans*	HDI	0.5	126
Micrococcus		TH13	1.2	300
Moraxella		AN24	0.55	136
Moraxella	*S. annandalei*	HD15	0.35	88
Corynebacterium		TH2	0.15	37
Micrococcus		AN2	0.55	136
Vibrio	*M. striata*	PHD14	0.75	189
Micrococcus		PTH24	0.92	228
Micrococcus		PAN1	0.45	111
Bacillus	*T. furcifera*	THD8	0.95	235
Moraxella		TDR	0.42	106
Moraxella		TAN6	1.00	250
Pseudomonas	*S. terebrans*	FG5	0.45	111
Micrococcus		MG2	1.40	350
Corynebacterium		HG1	1.20	300
Micrococcus	*S. annandaelei*	AFG26	1.15	286
Micrococcus		AMG34	0.66	165
Micrococcus		AHG25	0.72	180
Vibrio	*M. striata*	PFG30	0.54	134
Moraxella		PMG29	0.70	177
Bacillus		PHG8	0.75	189
Micrococcus	*T. furcifera*	TFG1	0.95	235
Moraxella		TMG15	1.15	2.86
Arthrobacter		THG25	0.82	204

Table 5. Cellulolytic activity in the mouth and viscera of various wood borers

Organsims	Source	Activity
S. annandalei	Mouth	140.00
	Viscera	55.00
S. terebrans	Mouth	214.00
	Viscera	190.00
M. striata	Mouth	204.00
	Viscera	134.00
T. furcifera	Mouth	180.00
	Viscera	134.00

enzyme (350µg glu.ml.$^{-1}$h^{-1}) activity. *Corynebacterium* from hind gut and *Micrococcus* from the exoskeleton of the same organisms showed enzyme activity of 300µg glu.ml^{-1} h^{-1}. However, *Corynebacterium* isolated from the exoskeleton of *S. annandalei* had less enzyme activity (37µg glu.ml.$^{-1}$h^{-1}) and feeble (0.15 OD) growth. Table 5 shows the cellulolytic activity in mouth and visceral regions of selected wood borers. Invariably in all the four wood borers, the mouth regions exhibited higher activity compared to viscera. Of these, the activity was maximum in the mouth of *S. terebrans* (214µg glu.ml^{-1}.h^{-1}). The visceral mass of *S. annandalei* had the least (55µg glu.ml^{-1}.h^{-1}) enzyme activity.

Cellulolytic activity is widespread in invertebrates where it is associated with gut flora or perhaps produced by the animals themselves. Sulfutile, a liquid formed during the preparation of cellulose from wood is also fermented by microorganisms. Cellobiose which is a product of the digestion of cellulose can be converted into glucose by the action of β-glucosidase (Fischer & Zempplen, 1910. A fungus, *Cladosporium cladosporoides* from soil showed maximum cellulase activity on carboxy methyl cellulose (CMC). This was mainly extracellular (Lynch et al., 1981). They also stated that large variations in cellulase activity was mainly because of assay substrate and location of the enzyme reserve.

The present investigation revealed that the wood borers by themselves are capable of secreting cellulase and perhaps the indigenous cellulolytic bacteria associated with wood borers help them in boring through the wood by breaking down the cellulose molecule.

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Enzymatic Profile and Antibiotic Sensitivity of Some *Vibrio* and *Aeromonas* Strains

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API-ZYM system and antibiotic sensitivity assay were used to characterize twelve strains of *Vibrio* and three strains of *Aeromonas*, each belonging to different species. API-ZYM showed that all tested strains exhibited negative activity for α-galactosidase, β-glucuronidase, α-mannosidase and α-flucosidase. All strains expressed positive activities for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and naphthol AS-BI phosphohydrolase. β-glucosidase activity was a characteristic of the three *Aeromonas* strains. Also, *Aeromonas* strains as well as *V. damsela* gave very high N-acetyl β-glucosamidase activities. *Aeromonas hydrophila, A. trota* and *V. hollisae* were susceptible to all 9 tested antibiotics, and C10 and C30 were active against all tested strains. API-ZYM system and antibiotic susceptibility profile gave additional information that can help in identifying and separating very close species of *Vibrio* and *Aeromonas*.

Key words: Vibrio, Aeromonas, API-ZYM system, antibiotic susceptibility

Several species of *Vibrio* and *Aeromonas* have been recovered at high rate from seafood samples (Wong et al., 1992). Under the genus *Vibrio*, eleven species are known to be pathogenic or potentially pathogenic to humans, and six of them *(V. choleroa, V. parahaemolyticus, V. vulnificus, V. hollisae and V. furnissii)* are recognized to cause foodborne diseases (Buck, 1998; Hackney & Dicharry, 1988). *Aeromonas* species are ubiquitous in the aquatic environment, and are known as gastrointestinal pathogens (Gelbart *et al.*, 1985).

After primary screening on selective media, further biochemical and serological tests are always sought to differentiate among the members of Vibrio and Aeromonas. Enzymatic profiling of microorganisms has been an additional and valuable method in microbial classification (Sakai *et al.*, 1993). The API-ZYM is a semi quantitative and rapid method that helps in studying the enzymatic reactions of microorganisms and has been used to study the enzymes of Gram positive and Gram negative bacteria (Velazquez & Feirtage, 1997; Kalogridou-Vassiliadou, 1992; Slade, 1992; Desjardins & Roy, 1990; Corral & Buchanan, 1990; Tzanetakis & Litopoulou-Tzanetaki, 1989; McKellar, 1986), yeast (Schmidt *et al.*, 1979; Schmidt & Lenior, 1980), and mold (Hsiao & Hsiao, 1998; Vagvology *et al.*, 1996; Bridge *et al.*, 1993).

Sensitivity to antibiotics is another tool that may be applied to differentiate among microorganisms. Moreover, certain antibiotics may be incorporated to culture media to make them more selective to resistant strains (Lentsnet *et al.*, 1980; Sakazaki *et al.*, 1986; Sloan *et al.*, 1992). In particular, several studies have focused on the antibiotic sensitivity of Vibrios (Pedersen *et al.*, 1995; Abraham *et al.*, 1997; Khin *et al.*, 1996; Koga & Takumi, 1996; Pradeep & Lakshmanaperumalsamy, 1985; Buogo *et al.*, 1980).

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The aim of this study was to characterize and compare different species of *Vibrio* and *Aeromonas* using their enzymatic profiles and antibiotic sensitivity patterns.

Materials and Methods

Vibrio hollisae ATCC 33563), *Vibrio vulnificus* (ATCC 27562), *Vibrio parahaemolyticus* (ATCC 17802), *Vibrio alginolyticus* (ATCC 17749), *Vibrio fluvialis* (ATCC 33809), *Vibrio mimicus* (ATCC 33653), and *Vibrio furnissi* (ATCC 35016) were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland, USA). *Vibrio anguillarum, Vibrio harveyi, Vibrio cholerae, Vibrio carchariae, Vibrio damsela, Aeromonas hydrophila, Aeromonas media* and *Aeromona trota* were isolated from fish samples and identified with the aid of API 20E Kit (bioMerieux sa, France) and the Biolog Microbial Identification System (GN plates, Biolog, Inc., California, USA). All strains were maintained on tryptic soy agar (TSA) containing 3% NaCl.

Microorganisms were grown in tryptic soy broth (TSB) containing 3% NaCl for 18 h at 30°C for *V. hollisae* and *V. vulnificus* and at 36°C for the rest of the microorganisms. Each culture was then lawned onto the surface of TSA plates supplemented with 3% NaCl. After 24 h incubation period, cells were suspended in physiological saline (0.85% NaCl). The turbidity was adjusted to correspond to MacFarland No.5 standard. The API-ZYM strip (bioMerieux sa, France) contains 19 microtubes of dehydrated chromogenic enzyme substrates and one microtube of buffer as a control. Each microtube was inoculated with two drops of the bacterial suspension, and was then incubated within its humidified chamber for 4 h at 37°C. After incubation, one drop of ZYM A and one drop of ZYM B reagents were added to each well. Color reactions were allowed to develop for 5 min, after which the enzyme activity level was determined in accordance with the colour chart supplied by the manufacturer. A value in the range between 0-5 was assigned to the developed color, where zero corresponds to a negative reaction, 1, for the liberation of 5 nanomoles, 2, for 10 nanomoles, 3 for 20 nanomoles, 4 to 30 nanomoles and 5 to 40 nanomoles of reaction product. Each API-ZYM result (of one strip) was scored as described by Sakai *et al.* (1993). The 19 enzymes were divided into 7 groups, each one composed of 3 enzymes, except the last group that contained only one enzyme (a-flucosidase). When the activity of the first enzyme in each group is positive, the score is 1. When the activity of the second enzyme

Table 1. Antibiotics used, concentration per disc, symbols and interpretative creiteria of inhibition zones

Antibiotic	Concentration or µg/disc	Symbol	Inhibition zone* (diameter mm)		
			Resistant	Intermediate	Susceptible
Chloramphenicol	10	C10	-	-	-
Chloramphenicol	30	C30	≤12	13-17	≥18
Amoxycillin	25	AML25	-	-	-
Nalidixic acid	30	NA30	13	14-18	19
Doxycyclin-HC1	30	DO30	16	17-18	19
Sulfamethoxazole	25	SXT25	10	11-15	16
Polymyxin B	(300)	PB300	8	9-11	12
Colistin sulfate	25	CT25	-	-	-
Tetracycline	30	TE30	14	15-18	19
Neomycin	30	N30	12	13-16	17

*Cited from Pradeep and Lakshmanperumalaswamy, 1985

is positive, the score is 2. When the activity of the third enzyme is positive, the score is 4. No score was given for negative reaction. The scores of each group are totalled and expressed as a seven-digit number.

For antibiotic sensitivity assay, all bacterial strains were activated (24 h) in TSB supplemented with 3% NaCl, and then inoculated onto the surface of Muller Hinton agar using sterile cotton swabs. The inoculum was allowed to dry for about 15 min and ten different antibiotic discs (Table 1) were aseptically placed on the surface of the inoculated agar (3-4 discs/plate). The plates were then incubated for 24 h at 30°C for *V. hollisae* and *V. vulnificus* and 37°C for the rest of tested microorganisms. The diameter of inhibition zone of each antibiotic was measured (mm) and recorded based on the sensitivity level (resistant, intermediate and susceptible).

Results and Discussion

Data (Table 2) indicate the absence of α-galactosidase, β-glucuronidase, α-mannosidase, and α-fucosidase in all tested *Vibrio* and *Aeromonas* strains. Besides other enzymes, these four enzymes had negative activity when tested in *A. sobria* and *A. hydrophila* (Gelbart *et al.*, 1985; Waltman II *et al.*, 1982) and in *V. vulnificus* (Biosca & Amaro, 1996). Similar results had been obtained by Sakai *et al.* (1993) except that some strains of *V. anguillarum* and *A. hydrophila* showed positive reactions with β-glucuronidase. All tested strains showed positive activities in respect of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Sakai *et al.* (1993) reported similar results, but there was one strain (out of 11) of *V. anguillarum* that showed negative reaction for acid phosphatase. Similar results were also obtained with *A. hydrophila* from different sources (Waltman *et al.*, 1982) and with *V. vulnificus* (Biosca & Amaro, 1996). Fous *et al.* (1993) reported similar enzymatic activity for *V. damsela* except for cystine arylamidase that gave weak (<5 nanomoles) reaction. The results also revealed that *A. media*, *A. hydrophila*, *A. trota* and *V. damsela* had very high (40 nanomoles) activity for n-acetyl-β-glucosamidase. Also, the results indicated that the three *Aeromonas* strains were the only ones among the tested strains showing positive β-glucosidase activity. Therefore, this enzyme can be used to distinguish between the selected strains of the two genera. *V. damsela* can be separated from the other Vibrio strains by its high activity of n-acetyl-β glucosamidase.

The code number (Table 2) given to each strain based on the enzymatic profile can be easily used to separate and identify the microorganisms to the genus level. However, at the species level, most strains were well separated except in 2 cases. *V. parahaemolyticus*, *V. alginolyticus* and *V. damsela* had the same code (7623020), but can be easily separated based on the differences in their enzymatic activities, where esterase lipase and trypsin showed higher values in *V. parahaemolyticus* compared to *V. alginolyticus*, which in turn, gave higher activity of n-acetyl-β glucosamidase. On the other hand, *V. damsela* is characterized by very high (40 nanomoles), n-acetyl-β glucosamidase activity and the absence of trypsin. Similarly, *V. anguillarum* and *V. furnissii* had the same code number (7613020). *V. anguillarum*, however, exhibited stronger reaction for esterase lipase (C8) and n-acetyl-β glucosamidase, and weaker reaction for acid phosphatase compared to *V. furnissii*. The results of this study and those reported by Sakai *et al.* (1993) prove the usefulness of the API-ZYM system and the derived strain-code number in identifying closely related species. This code was only based on positive or negative results of the reaction, ignoring the level of enzyme activity. It is suggested, therefore, that identification using such procedure should take an extra step, taking into account the level of enzyme activity. As a result, different codes will be obtained for very close strains. More tests

Table 2. Enzymatic activities of *vibrios* and *Aeromonas* strains by the API-ZYM method[1]

Microorganism	Enzyme[2]																				Identification code
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
V. hollisae	0	5	2	4.5	0	4.5	0	0.5	0	0	0.5	2	0	0	0	2	0	0	0	0	7213400
V. vulnificus	0	5	2	3	0.5	5	1.5	1.5	0	0	2	2	0	0	0	0	0	0.5	0	0	7713020
V. parahaemolyticus	0	5	2	4	0	4	0.5	0	5	0	5	2	0	0	0	0	0	0.5	0	0	7623020
V. alginolyticus	0	5	2	2.5	0	4.5	1	0	3.5	0	5	2	0	0	0	0	0	2	0	0	7623020
V. anguillarum	0	5	1	2	0	5	0.5	2	0	0	0.5	2	0	0	0	0	0	4	0	0	7613020
V. harveyi	0	5	2	3.5	0.5	4.5	1	0.5	3.5	4	5	2	0	0	0	0	0	0.5	0	0	7773020
V. cholerae	0	5	1.5	2.5	0	5	0.75	1.5	0	0	2	2	0	0.5	0	0	0	1.5	0	0	7613520
V. carchairae	0	5	2	3	0	4	0.5	1	4	3	5	2	0	0	0	0	0	2	0	0	7673020
V. fluvialis	0	5	1.5	2.5	0.75	5	1	0.5	0	0	5	2	0	0.75	0	0	0	2	0	0	7713120
V. mimicus	0	5	3	3.5	0	5	1	3	0	0	5	2	0	1.5	0	0	0	2	0	0	7613120
V. furnissii	0	5	0.5	0.75	0	5	1	2.5	0	0	2	2	0	0	0	0	0	1.5	0	0	7613020
V. damsela	0	5	2	4.5	0	4	0	0.5	0	0	2	2	0	0	0	0	0	5	0	0	7623020
A. media	0	3	2	4.5	0.5	4	0	0.75	0.5	0	2	2	0	3	0	0	1	5	0	0	7333130
A. hydrophila	0	5	2	4	1.5	2.5	0	0.5	1	0	1.5	2	0	0	0	0.5	4.5	5	0	0	7333430
A. trota	0	3	3	5	1	3	0	0	0.5	0.5	2	2	0	3.5	0	0	2	5	0	0	7323130

[1]Enzyme activity mark 0=0 nanmole, 0.5=<5 nanmoles; 1=5 nanmoles; 2=10 nanmoles; 3=20 nanmoles, 4=30 nanomoles and 5=≥40 nanomoles.

[2]Enzymes 1 = control, 2 = phosphatase alkaline, 3=esterase(C4), 4=esterase lipase (C8), 5=lipase(C14), 6=leucine arylamidase, 7=valine arylamidase, 8=cystine arlamidase, 9=trypsin, 10=chemotrypsin, 11=phosphatase acid, 12=naphthol-AS-phosphohydrolase, 13=α galactosidase; 14=β galactosidase; 15=β glucuronidase; 16=β glucosidase; 17=β glucosidase; 18=N-acetyl-b glucosamidase; 19=α mannosidase; 20=α fucosidase

Data are average of two experiments

Table 3. Antibiotic susceptibility of selected *Vibro* and *Aeromona* strain by disc diffusion method

Microorganism	Inhibition zone (mm) and status*										
	C10	C30	AML25	NA30	DO30	SXT25	PB300	CT25	TE30	N30	Gr.#**
V. hollisae	32 S	38 S	36	42 S	36 S	22 S	26 S	24	36 S	18 S	1
V. vulnificus	32 S	38 S	26	38 S	38 S	14 I	0 R	0	40 S	16 I	5
V. parahemolyticus	20 S	22 S	0	20 S	20 S	12 I	0 R	0	22 S	12 R	7
V. alginolyticus	20 S	24 S	0	18 I	22 S	14 I	10 I	10	22 S	12 R	6
V. anguillarum	24 S	28 S	16	26 S	24 S	20 S	14 S	16	30 S	12 R	3
V. harveyi	22 S	28 S	0	26 S	28 S	20 S	0 R	0	28 S	14 I	4
V. cholerae	26 S	32 S	24	24 S	28 S	22 S	10 I	8	30 S	16 I	2
V. carchairae	28 S	32 S	14	30 S	28 S	20 S	0 R	0	32 S	14 I	4
V. fluvialis	24 S	26 S	16	30 S	20 S	20 S	12 S	14	24 S	12 R	3
V. mimicus	20 S	26 S	16	26 S	26 S	18 S	14 S	12	26 S	12 R	3
V. furnissii	20 S	24 S	14	16 I	18 I	16 S	12 S	12	18 I	12 R	6
V. damsela	24 S	28 S	10	28 S	26 S	20 S	8 R	8	26 S	14 I	4
A. media	38 S	44 S	26	46 S	40 S	22 S	26 S	26	46 S	14 I	2
A. hydrophila	38 S	44 S	20	46 S	38 S	30 S	28 S	28	48 S	26 S	1
A. trota	36 S	42 S	14	42 S	36 S	22 S	18 S	22	40 S	24 S	1

*S = susceptible, I = intermediate; R = resistant

** Microorganism group, where # 1 is most susceptible and # 7 is most resistant

such as gram strain, catalase and oxidase activities can be included in the identification code as well.

Table 1 shows the antibiotics used, its concentration, and the interpretative criteria of inhibition zones (Pradeep & Lakshmanperumalsamy, 1985). There were no published data nor any provided by the manufacturer on the interpretative criteria for chloramphenicol (C10), amoxycillin (AML25), and colistin sulfate (CT25). However, the interpretative criteria for C30 were used for C10 since the differences in the inhibition zones between the two concentrations were very small and the results of C10 exceeded the susceptible limit ((18 mm) for the C30 interpretative criteria. Excluding the results of AML25 and CT25, it was noticed that *A. hydrophila, A. trota,* and *V. hollisae* were susceptible to all (eight) antibiotics (Table 3). Also, C10 and C30 were very effective against all tested organisms. On the other hand, neomycin (N30) was the least effective of the tested antibiotic where 20% of the cultures were susceptible. Although, doxycyclin-HCL (D030) and tetracycline (TE30) were very effective against most of the cultures, *V. furnissii* was the only species showing intermediate resistance against these two antibiotics.

Based on the inhibitory pattern of the different tested antibiotics, the tested microorganisms can be ascendingly arranged into seven groups according to their antibiotic susceptibility: 1) *A. hydrophila, A. trota* and *V. hollisae;* 2) *A. media* and *V. cholera;* 3) *V. anguillarum, V. fluvialis* and *V. mimicus;* 4) *V. carchariae, V. damsela* and *V. harveyi;* 5) *V. vulnificus;* 6) *V. alginolyticus* and *V. furnissii;* and 7) *V. parahaemolyticus.* This grouping may change when interpretative criteria for CT25 and AML25 are determined. More antibiotics and higher number of isolates should be investigated to get a more clear picture on using this assay to separate *Vibrio* species. It appears that polymyxin B, colistin sulfate, neomycin and amoxycillin can be easily used for such purpose.

It is evident from this study that both API-ZYM system and antibiotic susceptibility assay can serve as source of valuable additional information useful in identifying and characterizing members of Vibrio and Aeromonas. A modification in the API-ZYM system data interpretation is suggested to aid in separating very close species such as: *V. parahaemolyticus, V. alginolyticus* and *V. damsela.* Antibiotic susceptibility data indicated the possible use of some antibiotics such as polymixin B, colistin sulfate, neomycin and amoxycillin in developing selective media for isolation and identification of *Vibrio* species resisting these antibiotics.

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Use of Fermented Fish Ensilage in the Diets of *Etroplus suratensis* (Bloch)

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A feeding experiment was conducted to assess the ability of *Etroplus suratensis* (Bloch) to utilize diets containing fish ensilage as a major protein source. Attempt was also made to find out whether the neutralization of the fermented ensilage influenced the growth of *E. suratensis* or not. No significant difference in the survival rate of fish fed with various feeds was noticed (P(0.05). However, significant difference could be observed in specific growth rate,(P<0.01), food conversion efficiency and protein efficiency ratio (P<0.05). The study revealed that the diets containing fish ensilage, either neutralized with calcium hydroxide or without neutralization, could yield better weight gains (86.25% and 89.08% respectively) than other feeds. *E. suratensis* could utilize diets containing fish ensilage as the major source of protein for growth. While ensilage - based diet neutralized with sodium hydroxide recorded lower weight gains (80.87%) the effect was more adverse when potassium hydroxide was used as the neutralizing agent (56.18%).

Key words: Fermented ensilage, neutralization, feed, *Etroplus suratensis*, growth rate

Etroplus suratensis (Bloch), commonly called as pearl spot, is widely distributed in estuaries, backwaters and brackishwater lakes of India. Pearl spot is highly palatable and possesses an excellent nutritional profile with a good combination of all essential amino acids (Mukundan & James, 1978). Ability to utilize supplementary diets (Jayasinghe *et al.*, 1985) and rapid growth rate (Vijayaraghavan *et al.*, 1981) make *E. suratensis* an ideal species for culture.

Success of many commercial farming practices depends to a large extent on the development of low cost and nutritionally balanced diet. One of the most promising ways to reduce feed cost is to use fish ensilage as a major protein source in fish diets. Fish ensilage production is neither capital nor energy intensive when compared to fish meal production. The use of fish ensilage has been proved to be effective in the feeds of salmon (Crampton *et al.*, 1982; Jackson *et al.*, 1984) and in Indian major carps (Srinivasan *et al.*, 1982). The present study was undertaken (i) to assess the ability of *E. suratensis* to utilize fish ensilage as a major protein source in its diet, (ii) to examine whether the neutralization of the fermented ensilage is necessary or not in the feed preparation of *E. suratensis* and (iii) to elucidate the effects of use of various alkalis for the neutralization of silage on the growth of pearl spot.

Materials and Methods

Juveniles of *E. suratensis* collected from the brackishwater estuary, were brought to the laboratory and gradually acclimatised to freshwater over a period of 15 days. During the acclimatization period, the fish were fed with pellet diet containing 25% protein. Juveniles of uniform size of about 7 g were randomly stocked at the rate of 12 fish per tank in twelve circular plastic tanks each with a capacity of 50 litres.

Compressed air was continuously supplied through air stones to all the rearing tanks. Water samples were analysed every day for temperature), pH and ammonia nitrogen (APHA, 1995).

In the present study, fish ensilage was prepared by biological fermentation using minced silver belly (*Leiognathus* spp.). Molasses (15% of the mince) was used as carbohydrate source for the production of lactic acid through fermentation. Water was added (30%, v/w) as well as sodium chloride, (1%) and the mixture was made into a slurry and boiled for 30 min to inactivate endogenous enzymes involved in the hydrolysis of protein and then cooled to room temperature. A one day old fermented cabbage was added (5% of the minced flesh) to effect fermentation. The slurry was stored in plastic containers with air-tight lids and allowed to ferment at room temperature. The pH of the contents reached 4.4 in 48 h indicating sufficient fermentation. The ensilage was then dried at 60°C to bring down the moisture level to 10% for further use in the test feeds.

Six isonitrogenous (25%) feeds were prepared following the 'square method' (Hardy, 1980). While diet 1 was formulated to contain fish meal as the major protein source and served as control, diet 2 was formulated with ground nut oil cake as the major source of protein. The remaining four feeds (diets 3-6) were prepared using fish ensilage as the major protein source (Table 1). The dry ingredients were pulverized to fine particles and mixed thoroughly in a mixer until homogenous. The pH of these diets 4,5 and 6 was adjusted to 6.8±0.1 by adding the required quantity of sodium hydroxide (diet 4), calcium hydroxide (diet 5) and potassium hydroxide (diet 6) and mixed using distilled water. All the diets were made as dough of good consistency and were steam cooked for 10 min with intermediate stirring. They were then passed through a hand pelletiser to obtain pellets

Table 1. Percentage of ingredients and proximate composition of the experimental diets

	Test diets					
	1	2	3	4	5	6
Ingredients %						
Fish meal	40.00	-	12.70	12.70	12.70	12.70
Fish ensilage	-	-	27.50	27.50	27.50	27.50
Groundnut oil cake	-	62.00	-	-	-	-
Rice bran	43.00	21.00	42.80	42.80	42.80	42.80
Tapioca flour	10.00	10.00	10.00	10.00	10.00	10.00
Cod liver oil	1.00	1.00	1.00	1.00	1.00	1.00
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin mix[1]	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix[2]	3.00	3.00	3.00	3.00	3.00	3.00
Proximate composition (as % dry matter)						
Crude protein (N x 6.25)	25.21	25.23	25.17	25.01	24.98	25.20
Ether extract	7.76	8.60	7.20	7.30	7.18	7.25
Ash	15.45	9.23	15.30	14.80	15.10	15.20
Nitrogen free extract[3]	36.00	38.70	37.80	37.75	37.60	38.00
Estimated ME[4] (Kcal. $100g^{-1}$)	294.33	310.05	295.61	295.52	293.52	296.80

1 - Vitamin mix No.10 (New, 1987);

2 - Mineral mix No.5 (New, 1987);

3 - By difference;

4 - Metabolizable energy (ME) was estimated based on the physiological values of fish: Protein, 4.5 $Kcal.g^{-1}$; Carbohydrate, 3.3 $Kcal.g^{-1}$; fat 8.0 $Kcal.g^{-1}$; (Brett & Groves, 1979)

Table 2. Proximate composition of the ingredients used in the experimental diets

Ingredients	Moisture (%)	Protein (%)	Fat (%)	Nitrogen* Free extract (%)	Ash (%)
Fish meal	9.90±1.02	63.10±2.05	7.90±0.25	0.02	19.08±1.05
Fish ensilage (dried)	10.01±2.03	60.25±1.06	5.20±0.15	0.95	23.59±0.55
Groundnut oil cake	7.34±1.35	42.00±2.15	5.52±0.17	29.04	15.24±1.07
Tapioca flour	9.50±1.30	2.00±0.75	3.00±0.01	79.50	6.00±0.65
Rice bran	2.00±0.35	9.25±1.25	10.55±0.05	59.20	19.00±0.05

* By difference

of 3 mm dia. The pellets were air dried for 2 h and then oven dried at 70°C for 12 h to obtain a moisture content of approximately 8%. Each of the six diets was fed to duplicate groups of fish in a completely randomized design. The fish were fed three times a day (9.00; 12.00; 15.00 h) at the rate of 10% of the body weight per day. Fish in each tank were weighed individually once in 2 weeks and the quantity of feed was adjusted after each weighing. Mortality if any, was recorded every day and the amount of feed was adjusted accordingly. Everyday, before fresh feeding, the uneaten feeds were collected, oven dried, weighed and the actual weight of diet consumed in each tank calculated. The experiment was conducted for 70 days. Proximate composition of the feed ingredients and experimental diets were analyzed following the standard methods of AOAC (1980).

The average growth data of the duplicate groups of each treatment were used for statistical analysis. The weight gain, specific growth rate, feed conversion efficiencies, protein efficiency ratios and survival rates were calculated for the different feeds studied. The results were tested using one-way analysis of variance.

Results and Discussion

The proximate composition of the feed ingredients and the formulated feed is given in Table 2. The weight gain, specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER) and survival rate of *E. suratensis* fed with various experimental feeds are presented in Table 3. The highest weight gain (89.08%) was recorded for fish fed ensilage based feed which was neutralized with $Ca(OH)_2$ (diet 5). However, the weight gain was not significantly higher ($P>0.01$) than those given non-neutralized ensilage diet (diet 3) (86.25%) or fish meal based control diet (diet 1) (85.66%). While fishes fed ensilage based

Table 3. Percent weight gain, specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER) and survival rate of *Etroplus suratensis* fed various test diets for 10 weeks

Test diets	Average initial Weight (g) ± s.e	Average final weight (g) ± s.e	Percent weight gain* ± s.e	SGR* ± s.e	FCE** ± s.e	PER** ± s.e	Percent** Survival rate ± s.e
1	7.7402±0.073	13.743±0.218	85.66±1.12^{d}	0.884±0.009^{d}	0.256±0.015^{b}	1.025±0.060^{b}	95.84±4.17^{b}
2	7.7250±0.126	12.200±0.288	68.26±1.05^{b}	0.231±0.013^{ab}	0.924±0.052^{at}	91.67±8.34^{ab}	
3	7.7161±0.092	13.338±0.243	86.25±1.00^{d}	0.889±0.08^{d}	0.254±0.012^{b}	1.014±0.049^{b}	95.84±4.17^{a}
4	7.7192±0.074	13.008±0.168	80.87±0.41^{c}	0.246±0.004^{c}	0.246±0.019^{b}	0.982±0.076^{b}	91.67±0.00^{a}
5	7.300±0.105	13.297±0.237	89.08±0.53^{d}	0.910±0.004^{d}	0.270±0.016^{b}	1.081±0.064^{b}	95.84±4.17^{a}
6	7.233±0.075	11.297±0.141	56.18±0.33^{a}	0.637±0.003^{a}	0.164±0.006^{a}	0655±0.024^{a}	95.84±4.17^{a}

* Column means not sharing the same superscript are significantly different at 1% level
** Column means not sharing the same superscript are significantly different at 5% level

diet neutralized with NaOH showed significantly lower ($P<0.01$) weight gains (80.87%) than those fed control diet (diet 1), the lowest weight gain (56.18%) was recorded in fish fed ensilage based diet neutralized with KOH (diet 6) ($P<0.01$). The animals fed on plant protein diet registered low weight gains (68.26%) compared to the control and non-neutralized fish ensilage based diets.

The significantly higher ($P<0.01$) weight gains recorded in fish fed fish meal and ensilage based diets over those fed the diet containing plant protein indicates the need for animal protein in the diets of *E. suratensis*. This may be due to the fact that both fish meal (New, 1987) and fish ensilage (Jackson *et al.*, 1984) have a well balanced amino acid pattern, which is essential in the dietary protein for the synthesis of new proteins, while most plant proteins like groundnut oil cake contain inadequate levels of one or more of the essential amino acids, particularly methionine (Lim & Dominy, 1989).

Weight gains of fishes fed on diet 3 and diet 5 were on par with or slightly higher than those given the control feed (diet 1). This indicates the possibility of replacing fish meal with fish ensilage in the diet of *E. suratensis* either without neutralization or after neutralization with $Ca(OH)_2$. Similar results have been reported in several fish species including Indian major carps (Srinivasan *et al.*, 1985), salmon (Crampton *et al.*, 1982; Jackson *et al.*, 1984), catfish (Wee *et al.*, 1986) and rainbow trout (Stone *et al.*, 1989). It has been pointed out that the simpler peptides present in ensilage are easily assimilable by fish (Stone & Hardy, 1986).

The feed conversion efficiency (FCE) of fishes fed the various test feeds are given in Table 3. Although fish fed the ensilage based diet neutralized with $Ca(HO)_2$ showed the best feed conversion efficiency (0.27), it was not significantly higher ($P>0.05$) than those fed control feed (0.256), non neutralized ensilage feed (0.254) and ensilage neutralized with NaOH (0.246). The FCE of plant protein diet and ensilage diet neutralized with KOH was significantly lower than that of other feeds ($P<0.05$). The lowest FCE (0.164) was recorded for fish fed the ensilage diet neutralized with KOH. This value was even lower than that recorded for fish given feed containing only plant proteins (0.231).

Except the group fed the ensilage based diet neutralized with KOH, all others recorded almost similar FCE and PER values indicating the harmful effects of addition of KOH for neutralizing fermented ensilage in feed preparation. The higher FCE and PER values recorded in general for the silage based diets also reveal that *E. suratensis* can efficiently utilize the diets containing fish silage as a major protein source. The survival rates of fish fed the different test diets were generally high and ranged between 91.67% and 95.84% (Table 3). The small variations observed in the survival rates of the various groups did not seem to be related to the type of test diets used.

While neutralization of the ensilage using $Ca(OH)_2$ slightly improved its nutritional value than the non-neutralized silage diet, use of NaOH and KOH reduced the weight gains of fish to levels even lower than those recorded for fish fed the control and plant-protein diets. Although exact mechanism for the growth depression is not clearly known, a probable reason may be that KOH and NaOH affect the protein of the diet and hence lower its nutritional quality. The levels of calcium, sodium and potassium in fish diets are of particular significance since all these elements have important roles to play for the maintenance of life of fish. While calcium is a chief constituent of bones and selected tissues, sodium and potassium serve in vital functions by controlling osmotic pressures and acid-base equilibrium in fishes (Lall, 1989). Excess or inadequate levels of these ions in the diet may affect normal functioning of the body, an understanding of which demands further research in this aspect. However, at this stage it can be

concluded that fermented fish ensilage does not require neutralization to serve as protein source in the diet of *E. suratensis* although neutralization with calcium hydroxide slightly improves its nutritional value.

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Effect of Dietary Lipid Levels on Growth and Survival of Fingerlings of the Indian Major Carp *Cirrhinus mrigala*

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Six isonitrogenous semi purified diets with graded levels of lipids (4.5, 6, 7.5, 9, 10.5 and 12%) were prepared in pelleted form and fed to the fingerlings of *Cirrhinus mrigala* for 60 days to evaluate the changes in growth and feed conversion. Absolute, relative and specific growth rates were found to be higher and FCR values better in fishes receiving a diet containing 7.5% lipid

Key words: Indian major carp, *Cirrhinus mrigala*, lipid requirement

In fish culture operations, the highest recurring cost is that of the feed (De Silva, 1988). Artificial feeding is an integral part of managed fish culture practices, where the focus is on maximum fish production with minimum feed cost (Jeyachandran & Raj, 1976). Although the technique of carp culture has been standardized to some extent in India, there is ample scope for further improvement through better management practices and optimization of quantity of nutrients to be given through artificial feeds. The traditional artificial diet of Indian major carps consisting of 1:1 mixture of oil cake and rice bran is not nutritionally balanced (Das & Ray, 1991). There is need to understand the basic nutritional requirements of each species in order to formulate cheap and nutritionally balanced diets. The reduction of protein level in the feed formulation and increase in the dietary lipid level to compensate the protein for energy requirement not only increases the fish growth but also reduces the feed cost from economic point of view (Phillip *et al.*, 1969). Dietary lipid is a major source of energy in fish feeds and it has protein sparing action (Sargent *et al.*, 1989; Ellis & Reigh, 1991). Dietary lipids supply energy and provide essential fatty acids needed for structural maintenance of membranes and proper functioning of many physiological processes. Lipids are almost completely digestible by fish and seem to be favoured over carbohydrate as an energy source (Ellis & Reigh, 1991). The present study was, therefore, designed to provide some information on the growth and feed conversion of fingerling of *Cirrhinus mrigala* at varying dietary lipid levels.

Materials and Methods

C. mrigala fingerlings were purchased from a private aquafarm near Ambasamudram, Tamil Nadu and acclimated to the laboratory condition. Six isonitrogenous diets containing 4, 5, 6, 7.5, 9, 10.5 and 12% of lipid (Table 1) were prepared in pelleted form using cod liver oil as the source of lipid. Fish from the acclimated lot were randomly allocated to experimental troughs (15 l capacity) containing 5 l of tap water, in triplicate groups of 6 fish for each diet. Thus, there were 6 treatments with 3 replicates for each lipid level. The feeding trial was conducted for 60 days. The fish were fed at 5% of body weight per day and the ration was divided into two doses (10 h and 16 h). Uneaten food

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Table 1. Proximate composition of experimental diets of *C. mrigala*

	Feed					
	1	2	3	4	5	6
Crude protein,%	35.38	35.06	34.92	34.56	34.54	34.47
Crude lipid, %	4.67	6.13	7.57	8.97	10.36	11.91
Crude carbohydrate, %	23.24	22.35	22.23	21.51	21.40	20.37
Energy kcal/ 100 g* (Calculated)	318.22	340.25	352.09	359.89	371.98	381.48

* Gross energy was calculated based on ADCP (1983). Protein: 5.5 kcal.g^{-1}, Carbohydrate: 4.1 kcal.g^{-1}, Fat: 9.1 kcal.g^{-1}

was removed and weighed to calculate the amount of feed taken. Constant aeration was provided to the experimental fish along with 50% water exchange daily. The water quality parameters during the feeding trial were found to be temperature 29±1.5°C; pH 7.2 to 8.1 and dissolved oxygen 4.2 to 5.4mg.l^{-1}. Fortnightly weight gain was measured and absolute, relative and specific growth were calculated (Rangaswamy *et al.*, 1998). Proximate composition of diets was estimated (AOAC, 1984). Gross energy of the diets was calculated based on the values suggested by ADCP, (1983). Data were statistically analysed using Analysis of Variance test (Pillai & Sinha, 1968).

Results and Discussion

The effect of dietary lipid levels on the growth and survival of *C. mrigala* fingerlings for 60 days is presented in Table 2. It is clear from the data that absolute (0.602 g), relative (301%) and specific growth (2.31%) were higher and the FCR (1.25) better in fish which received a diet containing 7.5% of lipid. Hence it may be concluded that 7.5% of lipid in diet appears to be optimum for the maximum growth of C. mrigala fingerlings. The earlier reports on fish such as channel catfish *(Ictalurus punctatus)* (Dupree, 1969); rainbow trout *(Salmo gairdneri)* (Watanabe *et al.*, 1979) and red drum *(Sciaenops ocellatus)* (Williams & Robinson, 1988; Ellis & Reigh, 1991) indicated a dietary lipid requirement of 6-11%. The ability of fish to use lipid as a major source of energy has been investigated in many fish species (Watanabe, 1982; Sargent *et al.*, 1989; Sin, 1973; Singh, 1990 and Jafri *et al.*, 1995). It was found that the dietary lipid requirement of Indian major carp falls between 4 and 7% and that of tilapia between 4 and 9% (Singh, 1991; Hanley, 1991), whereas in the present study the dietary lipid requirement of *C. mrigala* was 7.5%. Growth of fish at different dietary lipid levels was significantly different ($p<0.01$). However, The growth at 9, 10.5 and 12% levels of lipid was comparable (Table 2) which suggested that incorporation of lipid in diets beyond 7.5% might not be advantageous in enhancing growth. Jafri *et al.* (1995) reported that feeding higher levels of lipid to *C. mrigala* resulted in reduced growth. Similar phenomenon was observed in catfish *Clarias batrachus* (Anwar & Jafri, 1994). Studies on channel catfish, striped bass and red drum suggested that feeding higher levels of lipid either reduced the growth or did not improve it (Dupree, 1969; Williams & Robinson, 1988; Patricia *et al.*, 1993).

Table 2. Growth performance of *C. mrigala* fed on varying levels of dietary lipids for 60 days

Lipid level (%)	Initial mean weight (g)	Final mean weight (g)	Absolute growth (g)	Relative growth (%)	SGR	FCR	Survival
4.5	0.2	0.631	0.431	215.5	1.91	2.99	83.3
6.0	0.2	0.645	0.445	222.5	1.95	2.32	100.0
7.5	0.2	0.802	0.602	301.0	2.31	1.25	100.0
9.0	0.2	0.738	0.538	269.0	2.17	1.65	100.0
10.5	0.2	0.687	0.487	243.5	2.04	1.98	100.0
12.0	0.2	0.621	0.421	210.5	1.88	2.16	83.3

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Socio-Economic Status of Marine Fishermen in Two Fishing Villages of Orissa

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The socio-economic status of fishermen in two marine fishing villages of Orissa State viz., Pentakota and Belinoliasahi were measured using a Socio-economic Status Index (SESI) developed in the study. It was seen that the mean SESI scores of respondents in these two villages (57.53%) had differed significantly at 1% level. Of the nine regression co-efficients calculated between the SES scores and independent variables, the regression co-efficient of two variables viz., knowledge about technological practices and sources of information used were found to be positive and significant in Pentakota village and the R^2 (73.38%) was highly significant. But in Belinoliasahi, the R^2 (28.30%) was not significant. The results also revealed that the fishermen had favourable attitude towards the motorisation of fishing crafts (71.60% and 81.56%) in both the villages.

Key words: Socio-economic status, fishermen, Orissa

Inspite of large scale awareness about fishery technological practices, fishermen could not adopt costlier innovations due to socio-economic constraints (Balasubramaniam *et al.*, 1991). In a fishing village, eventhough, the fishermen have more or less a common life style and social background, the ownership pattern of fishing craft and gear, and individual status vary. Considering the non availability of a precise method to measure the socio-economic status of fishermen, the present work was undertaken with the following specific objectives: i) to delineate the various components and measure the socio-economic status of marine fishermen in the selected fishing villages ii) to determine the role of the selected variables in influencing the socio-economic status of fishermen and iii) to assess the relationship between the selected variables and attitude of fishermen towards motorisation of traditional crafts.

Materials and Methods

In order to measure the socio-economic status, twenty components were delineated by examining the literature and through pre-testing. Then, 35 experts were requested to assign weightages/percentages scores to these 20 components in such a way that the total of these 20 weightages comes to 100. While assigning weightages, the importance of each component as an indicator of socio-economic status was considered by the experts. Based on the responses, the arithmetic means of components were calculated to obtain the final weightages of 20 components. These components were categorized into three major categories viz., personal factors, social factors and economic factors. These categories and the weightages of 20 components are given in Table 1.

The weightages assigned to 20 components were further divided among the different rating scale categories in each component, based on the experience and expertise of five experts. The reliability coefficient of the socio-economic status index was calculated by the test-retest method (Guilford, 1984) and was found to be high (0.933). The validity of the index was ensured by content validity.

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Table 1. Weighted components of socio-economic status index of fishermen of two of fishing villages in Orissa

Components	Weightages (%)
Personal factors	
Experience	15
Education	6
Subsidary occupation	4
Marital status	2
Information sources used	3
Type of personality	3
Peer group communication	3
Sub total	**36**
Social factors	
Type of family	3
Caste	3
Social participation	4
Social group affiliation	4
Adherence of social values & laws	3
Sub total	**17**
Economic factors	
Crew engaged	4
Land owned	5
House owned	5
Material possession	5
Investment on craft	8
Investment on nets	7
Investment on engine	6
Annual income	7
Sub total	**47**
Grand total	**100**

For measuring the attitude of fishermen towards motorization of traditional crafts, an attitude scale was developed based on the equal appearing interval method (Thurstone & Chave, 1929) with slight modifications (Edwards, 1969). The reliability of the scale was determined by test-retest method and the validity of the scale was ensured by content validity.

Independent variables such as risk preference, information need, credit orientation, cosmopolitan/local nature, technological adoption and sources of information were measured through different rating scales and then, corresponding index scores were calculated. High score on the cosmopolitan nature referred to the tendency of an individual to be in contact with those outside his own community while lower score on the index referred to the local nature which meant the confinement of an individual within his own community. Knowledge about technological practices was measured through a teacher test developed for the purpose. Interview schedules were used for collecting the data from the random samples of 50 fishermen in each of the two fishing villages selected viz., Pentakota and Balinoliasahi in Orissa State during the year 1995-96.

Results and Discussion

The socio-economic status index (SESI) mean scores of fishermen respondents in the two fishing villages are given in Table 2. The results revealed that the mean SESI score of fishermen in Pentakota village was higher (57.52%) than the mean index score of fishermen in Balinoliasahi village (47.55%) and the socio-economic status difference between the fishermen in these two villages were found to be highly significant at 1 per cent level.

It could be seen that the fishermen of Pentakota village had significantly higher mean scores on all the three major components viz., personal factors, social factors and economic factors than the fishermen of Balinoliasahi village.

It was also observed that due to the operation of marine plywood boats with outboard engines, synthetic fishing nets and, technological advancement was clearly visible in Pentakota than in Balinoliasahi village where the catamarans and other non motorized crafts were more predominant. Thus, it is evident that technological advancement of a fishing village such as Pentakota would also result in overall significant progress on the socio-economic status of fishermen (mean ESI = 57.52% in Pentakota and 47.55% in Balinoliasahi).

Table 2. Socio-economic status index (SESI) scores of fishermen in two fishing villages in Orissa

Components	Scores assigned	Scores obtained				
		Pentakota (n_1:50)		Balinoliasahi (n_2:50)		
		$\bar{X}$	SD	$\bar{X}$	SD	
Personal factors	36.00	19.82 (55.05%)	3.34	18.30 (50.83%)	3.54	2.36*
Social factors	17.00	10.52 (61.88%)	1.87	9.84 (57.88%)	0.68	2.54*
Economic factors	47.00	27.38 (58.25%)	5.05	19.41 (41.29%)	3.68	9.38**
Total	100.00	57.52	8.51	47.55	5.89	8.03**

* Significant at 5% level; **significant at 1% level
Figures in parathesis indicate percentage of assigned scores

When the mean scores of these factors were expressed in terms of percentages of assigned weightage, it was seen that in Pentakota village, the scores obtained were 61.88% of the maximum possible on social factors, 58.25% of the maximum possible on economic factors and 55.05% of the maximum possible on personal factors. Similarly in Balinoliasahi village also, the scores obtained were 57.88% on social factors, 50.83% on personal factors and 41.29% on economic factors. Thus, in both villages there was further scope for improvement in their social, personal and economic conditions.

The mean scores and standard deviations of nine selected variables of fishermen in the two fishing villages are given in Table 3. The results revealed that there are significant differences between the fishermen of Pentakota and Balinoliasahi villages on all variables except cosmopolitan/local nature. Of the eight differentiating variables, the fishermen of Pentakota had higher mean scores on four variables viz., knowledge about technological practices, technological adoption, sources of information, and credit utilization. The fishermen of Pentakota had lower mean scores on four variables viz., risk preference, information need, attitude towards motorization of crafts and number of fishing days per day. On an average, Pentakota had higher technological adoption, higher knowledge about technological practices and used more communication channels besides increased credit utilization.

Due to higher investments and comforts in existing fishing crafts, it was observed that they had preferred lesser risks than the fishermen of Balinoliasahi village. Further, average number of fishing days per year was higher for the fishermen of Balinoliasahi as they could operate their non-motorized

Table 3. Mean scores (%) and standard deviations of selected variables in two fishing villages in Orissa

Variables	Pentakota (n_1=50)		Balinoliasahi (n_2=50)		't'
	*	SD	*	SD	
Risk preference	57.95	12.05	81.35	4.77	14.38**
Information need	42.71	14.19	49.60	5.20	3.17**
Attitude towards motorization of crafts	71.60	3.68	81.56	6.87	9.15**
Credit utilization	85.76	6.36	9.33	25.52	4.32**
Cosmopolitan-local nature	73.27	29.91	81.86	21.63	1.97
Knowledge about technological practices	83.30	5.94	72.50	4.43	9.78**
Technological adoption	64.80	16.44	43.20	16.34	6.14**
No. of fishing days in a year	268.60	30.10	325.60	16.18	11.55**
Sources of information used	22.31	6.74	15.52	5.15	5.51**

**Significant at 1% level

crafts like catamarans without much operational expenses. The results further revealed that the fishermen had favourable attitude towards the motorisation of fishing crafts in both the villages ($\overline{X}_1$ = 71.60; ($\overline{X}_2$ = 81.56) and the fishermen in Balinoliasahi had shown more positive attitude than in Pentakota village.

The correlation and regression coefficient calculated between the socio-economic status and the selected independent variables are given in Table 4. It was seen that in Pentakota village, information need, cosmopolitan/local nature technological adoption and sources of information were found to have positive and significant

Table 4. Correlation and partial regression co-efficient of socio-economic status indices and selected varibales.

Variables	Pentakota (n_1:50)				Balinoliasahi (n_2:50)			
	Cor. Coeffts	Partial reg. Coeffts	Std. Error	't'	Cor. Coeffts.	Partial reg. Coeffts.	Std. error	't'
Risk preference	-0.299*	-0.055	0.066	0.834	-0.041	-0.099	0.184	0.535
Information need	0.436*	0.054	0.061	0.880	0.287*	0.354	0.194	1.825
Attitude towards motorization of crafts	0.146	0.154	0.196	0.890	-0.275	-0.375	0.125	3.004**
Credit utilization	-0.059	-0.017	0.127	0.131	0.200	0.044	0.035	1.253
Cosmopolitan / local nature	0.511*	0.045	0.029	1.575	-0.035	0.019	0.042	0.459
Knowledge about technological praticies	0.238	0.371	0.126	2.938**	-0.216	-0.270	0.205	1.314
Technological adoption	0.474*	0.037	0.056	0.657	-0.139	0.178	0.062	0.289
No of fishing days in a year	0.215	0.006	0.026	0.216	-0.032	0.013	0.055	0.229
Sources of information used	0.791**	0.764	0.143	5.388**	0.070	-0.032	0.164	0.192

* Significant at 5% level R^2 = 0.734; F=12.256*
** Significant at 1% level R^2 = 0.283; F = 1.754

Table 5. Attitude towards motorization of crafts and influence of selected variables in two fishing villages in Orissa

Variables	Pentakota (n_1:50)				Balinoliasahi (n_2:50)			
	Cor. Coeffts	Partial reg. Coeffts	Std. Error	't'	Cor. Coeffts.	Partial reg. Coeffts.	Std. error	't'
Risk preference	0.018	0.110	0.053	0.209	-0.299	-0.347	0.204	1.701
Information need	0.093	0.034	0.049	0.697	0.280*	0.376	0.223	1.687
Credit utilization	0.023	0.027	0.101	0.268	0.132	0.040	0.040	1.005
Cosmopolitan / local nature	0.581	0.006	0.024	0.269	0.040	0.037	0.048	0.774
Knowledge about technological practices	0.211	0.109	0.110	0.993	-0.157	-0.249	0.236	1.054
Technological adoption	0.032	0.010	0.045	0.216	-0.010	0.008	0.071	0.118
No. of fishing days in a year	-0.013	-0.010	0.0205	0.508	-0.064	0.007	0.063	0.115
Sources of information used	-0.003	-0.155	0.147	1.053	0.051	-0.072	0.187	0.383
Socio-economic status	0.146	0.111	0.125	0.900	-0.275	-0.490	0.163	3.004**

R^2 = .095; F = 0.467 R^2 = .312; F = 2.012

correlation with the socio-economic status of fishermen. 'Risk preference' was found to be negatively correlated with the socio-economic status. Earlier studies reported that in agriculture, the farmers with higher socio-economic status were found to prefer risks in innovative farming (Rogers & Shoemaker,1971; Singh, 1982; Juliana *et al.*, 1991). However, among the fishermen, socio-economic status and risk preference were found to be negatively correlated, probably, due to the differences in the nature of occupation.

Only two variables viz., the knowledge about technological practices and sources of information, had shown significant regression coefficient among the fishermen in Pentakota village. When all variables were taken together, they had accounted for 73.38% of the variation on the socio-economic status scores of fishermen and the R^2 value was found to be highly significant. In Balinoliasahi village, it was seen that the independent variables had accounted only 28.30% of the variation on the dependent variable and the R^2 was found to be non significant. It could be interpreted that as the knowledge, technological adoption and number of information sources were more positively associated with the socio-economic status, the fishermen in the higher socio-economic category would have to be convinced initially and their participation would positively influence others. Rogers & Shoemaker (1971) also reported that the elites in the clientele system acted as the gatekeepers while the change agents introduced innovations.

The influence of selected variables on the attitude of fishermen towards the motorization of fishing crafts is given in Table 5. The results revealed that in Pentakota village, none of the variables had yielded significant correlation and regression coefficients. The R^2 was found to be low (0.095) and non significant. In Balinoliasahi village, when there was an increase in the socio-economic status, there was corresponding decrease in the attitude score. Probably, this might be one of the reasons for the predominance of non monotrized crafts in Balinoliasahi among all the categories of fishermen. R^2 was found to be low (0.3116) and non significant in this case.

Thus, the study revealed that precise measurement of socio-economic status of fishermen in fishing villages will indicate the less developed components in each village and they can be taken as the bench mark levels before initiating development programmes.

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An Economic Appraisal of Freshwater Prawn Farming in Thanjavur District

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An economic appraisal of freshwater prawn farming was made by conducting a field survey from January to April 2000, among 25 randomly selected freshwater prawn farmers in Thanjavur district of Tamilnadu. Since the farm area of the selected respondents varied from 1.0 ha to 7.5 ha, the economic measures discussed in the results were computed for one-hectare farm area. The economic estimates indicated the economic feasibility of freshwater prawn farming. The net returns per ha per crop was found to be Rs.47,378 and the rate of return to investment was 45.34%.

Key words: Economic appraisal, rate of return, prawn farming, Thanjavur

Freshwater prawn farming is gaining importance in Tamilnadu in the wake of a legal ban on coastal shrimp farming because of obvious environmental and social implications. Availability of freshwater prawn seeds from natural resources combined with facilities for artificial seed production supports freshwater prawn farming to a large extent. The higher profitability of freshwater prawn farming vis-_-vis freshwater carp farming has been reported by Kumar *et al.* (2000) based on short term economic or statistical analysis. They estimated the production of freshwater prawn, *Macrobrachium rosenbergii* as 973 kg per ha per crop, compared to the average production of 3500 kg per ha per crop in the case of carp farming. The growing demand and the high export prices of prawns make freshwater prawn farming more profitable than carp farming. Information on the economics of freshwater prawn farming is limited and only a few studies have been conducted. Mahapatra *et al.* (1994) estimated the freshwater prawn production in polyculture systems along with carps at 1016 kg per ha per crop in the Sunderbans area of West Bengal State. Thus it becomes necessary to assess the economic feasibility of freshwater prawn farming for providing information relating to enterprise budgeting and preparation of bankable projects for the entrepreneurs before they take up or expand this venture.

Materials and Methods

Thanjavur district (Tamil Nadu), where the farming of freshwater prawn is gaining popularity, was selected to assess the economic feasibility of freshwater prawn farming. The size of sample of freshwater prawn farmers was fixed as 25. Random sampling was followed for the selection of respondents for the field survey. Thus the estimates are based on a field survey among 25 freshwater prawn farmers, conducted from January to April 2000. The size of farm operated by the farmers varied from 1.0 ha to 7.5 ha. The recorded data were therefore, not comparable as such. Hence, the data collected on capital investment, production cost, yield of prawns and total returns were tabulated respondent-wise and analysed. The average values per hectare were calculated for comparison and preparing standard enterprise budget. The economic analysis was carried out for the situations given below. The economics was studied for the areas where there was continuous supply of water and rearing of two crops in a year was

possible. The stocking density was 20,000 per ha for prawns and 850 per ha for carps. The land area used for aquaculture was assumed as leased with the rent treated as an item of fixed cost. Commercial feeds were used that would give 30-40% protein, 7% fat and 3% crude fibre. The survival rate considered was 70% for prawns and 80% for carps.

Results and Discussion

The items of capital investment required per hectare area for freshwater prawn farming is presented in Table 1. The average capital investment was Rs.2,09,000. Capital investment included excavation of ponds, establishment of bore well with submersible pump, paddle wheel aerators (2 nos), construction of semi-permanent store room and others. The average fixed cost per annum for 1 ha area was Rs.53,315 which included rent value on land, depreciation, interest on capital investment and expenses towards repairs and maintenance. The percentage contributions of interest on capital investment, depreciation, rent on land and expenses towards repairs and maintenance to total fixed cost per annum were 62.72, 22.27, 9.38 and 5.63, respectively. The items of production cost incurred per hectare are presented in Table 2. The total variable cost per crop with a duration of 6 months was estimated as Rs.84,465 (Table 3). The variable cost comprised feeds and feeding, management, seeds and stocking, wages and

Table 1. Capital investment for freshwater prawn farming

Items	Rs./ha
Excavation of ponds and formation of bunds (3600m^3 @ Rs. 20 m^3)	72,000
Formation of borewell (250 ft)	10,000
Cost of 5 hp submersible pump (1 no.)	20,000
Sluice gates for 4 ponds	20,000
Cost of paddle wheel aerators (2 nos.)	40,000
Construction of semi-permanent store room (10x10 ft)	35,000
Fishing nets and accessories	12,000
TOTAL	2,09,000

Table 2. Production costs in freshwater prawn farming

Items	Rs./ha
I. Fixed cost	
Rent on land per annum	5,000
Interest on capital investment of Rs. 2, 09,000 @ 15% per annum	33,440
Depreciation on farm equipments per annum	11,875
Expenses towards repairs and maintenance	3,000
Sub Total	53,315
II. Variable Cost	
Pond preparation	
Lime application 300 kg @ Rs.2/Kg	600
Application of urea 10 kg and super phosphate @ 25 kg	700
Provision of hideouts using palmyrah leaves	350
Cost of freshwater prawn seeds of 16,000 nos @ Rs.0.60 per seed	9,600
Cost of carp seeds of 650 nos. @ Rs. 1.00	650
Nursery feed for 370 kg @ Rs. 29 per kg	10,730
Shrimp feed (grower + fisher) for 960 kg @ Rs.25 per kg	24,000
Application of growth promoters mixed with feed	5,435
Electricity cost for water exchange	10,000
Wages for hired labour	4,000
Salary towards permanent watch and ward	6,000
Cost of lime applied at the time of water exchange	11,600
Total variable cost per crop	84,465
Total variable cost per annum (2 crops)	1,68,930
Total cost of production (for 2 crops)	2,22,245

Table 3. Components of total varibale cost in freshwater prawn farming

Items of cost	Rs./ha %
Feeds and feeding	40.165 (47.55)
Management	21,600 (25.57)
Seeds and stocking	10,250 (12.14)
Wages and salaries	10,000 (11.84)
Pond preparation	2,450 (2.90)
TOTAL	84,465 (100.00)

salaries and pond preparation and the corresponding percentage to total variable cost were 47.55, 25.57, 12.14, 11.84 and 2.90. The total variable cost per annum came to Rs.1,68,930. The total cost for 2 crops per annum was calculated to be Rs.2,22,245. The

Table 4. Returns from freshwater prawn farming

Items	Rs./ha
Sale of male freshwater prawns of 560 kg (legless) @ Rs. 225	1,26,000
Sale of female freshwater prawns of 196 kg @ Rs. 125	24,500
Sale of soft prawns for 15 kg @ Rs. 100	1,500
Gross returns per crop	1,52,000
Gross returns per annum (for 2 crops)	3,04,000
Returns through sale of fish of 520 kg @ Rs. 25 per kg	13,000
Total gross returns per annum	3,17,000

percentage shares of fixed cost and variable cost to total cost came to 23.99 and 76.01, respectively.

The average gross returns realized from 1 ha area is presented in Table 4. The average gross returns per crop was Rs.1,52,000 and Rs.3,04,000 for 2 crops per annum and the returns realized through the sale of fishes was Rs. 13,000. Thus the gross returns per annum for 2 crops of prawn farming was worked out as Rs.3,17,000. The corresponding total cost of production per annum was Rs.2,22,245. Thus the average net returns per annum worked out to Rs.94,755 with Rs.47,378 for one crop of prawn culture.

The economic measures estimated are presented in Table 5. The rate of return on capital investment was 45.34% per annum, an impressive and realistic rate. The input - output ratio i.e., the ratio between total

Table 5. Economic measures of freshwater prawn farming

Items	Rs./ha
Total annual gross returns (for 2 crops)	3,17,000
Total production cost per annum	2,22,245
Net returns per annum on total cost basis	94,755
Net returns per annum on total variable cost basis	1,48,070
Rate of returns on capital investment	45.34%
Input-output ratio	1:1.70
Cost of production per kg of prawns	144
Mean gross returns realized from sale of 1 kg of prawns (excluding fin fish)	197
Revenue realized per unit of sale of prawns	53

costs and total returns was found to be 1:1.70 which means that for every rupee of total cost incurred, about 70 paise of net profit could be earned. The cost of production per kg of prawns was estimated as Rs.144 whereas the mean gross returns realized per kg of sale was Rs.197 indicating a margin of Rs.53 per kg of prawns.

The investigation shows that the freshwater prawn farming in Thanjavur District, Tamil Nadu is an economically feasible project.

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Relative Effectiveness of Selected Extension Teaching Methods and Their Combinations in Teaching Rural Aquaculturists

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The present study revealed that among three individual extension methods (personal contact, group discussion, bulletin), personal contact was most effective in convincing the rural aquaculturists about the utility of an innovation. Bulletin occupied the last position in the order of effectiveness. Among the combinations of two, personal contact and group discussion resulted in maximum conviction regarding the merit of a new idea. The combination of group discussion and bulletin appeared to be least effective. The combination of personal contact, group discussion and bulletin proved significantly more effective than any of the three extension methods either individually or in combinations of two. The effectiveness of extension methods and their combinations was influenced by the level of education of the people. It may be concluded that in making aquaculture innovations popular with rural aquaculturists, better results can be achieved if the innovations are first exposed to the fish farmers with high level of education who can act as key communicators and multiply extension efforts.

Key words: Extension methods, aquaculture

The most important role of aquaculture extension workers is to communicate useful and practical research findings to fish farmers in such a way that they understand, accept and adopt these to attain a status of social and economic well-being. Adoption is a complex decision-making process involving a sequence of thoughts and actions. Before an individual tries out an innovation or actually adopts it, he weighs its merits in terms of his own situation. Unless fish farmers are convinced about the utility of an innovation in their own situation, there is little scope for its adoption. It is essential, therefore, that aquaculture extension workers use such extension teaching methods which help people make correct evaluation of an innovation. The present research project was designed to study the relative effectiveness of personal contact, group discussion bulletin and their combinations on fish farmers and to assess the effectiveness of these teaching methods.

Materials and Methods

The study was carried out with twenty farmers in two villages namely Deopar and Mahmada of Community Development Block, Pusa of Samastipur District in Bihar. Out of ten respondents from each village, five belonged to high education group (graduates) and five to low education group (non-graduates). The innovation selected was induced breeding of Indian major carps. The following extension teaching methods and their combinations were used: (i) personal contact, (ii) group discussion, (iii) bulletin, (iv) personal contact + group discussion, (v) personal contact + bulletin; (vi) group discussion + bulletin and (vii) personal contact + group discussion + bulletin.

Each of the seven selected extension teaching methods and their combinations was exposed to two villages, and one village (Malinagar) was taken as control where no extension teaching method was used. The

data on the respondents, (fish farmers) attainment of evaluation and conviction stage were collected sixty days after initial exposure to the innovation using the selected methods and their combinations. The behavioural characteristics enunciated by Singh (1965) were considered to determine whether a respondent had reached the evaluation or conviction stage. A three-point conviction scale was developed to study the respondents' evaluation of the innovation of induced breeding technique. For this, a large number of items reflecting favourable and unfavourable opinions about induced breeding technique were initially collected. These items were carefully scrutinized to eliminate vague and duplicate ones. Finally, five positive and five negative items were selected. Thus, the conviction scale consisted of ten items. For each item in the scale, the respondents were asked to select any one of three response categories: agree, undecided, or disagree. For positive items, the agreed response was given a weight of one and the disagreed response was given a weight of minus one. In case of negative items, the scoring system was reversed, with the disagreed response given a weight of plus one and the agreed response a weight of minus one. The total score of a respondent reflected the nature and intensity of his overall conviction about the utility of induced breeding technique. The total score was worked out by adding the scores of the individual items.

The total conviction scores of the individual respondents were subjected to analysis of variance (ANOVA) to see if there was any difference in their degree of conviction about the utility of induced breeding technique due to the selected extension methods and their combinations and levels of education.

Results and Discussion

The results of analysis of variance of conviction scores are presented in Table 1. The 'F' value for extension methods and their combinations was found to be highly significant. This reflected significant variation in the degree of conviction of the respondents who were exposed to the innovation through different extension methods and their combinations, irrespective of their levels of education. The F value for education was also found to be highly significant, indicating that the respondents having high and low level of education differed significantly in respect of their degree of conviction about utility of the innovation, irrespective of the extension methods and their combinations through which they were educated. This situation may be explained in terms of better ability on the part of the respondents belonging to the high education group to weight an innovation based on their specific farm situations.

Table 1. Analysis of variance of conviction score due to extension methods and levels of education

Source of variation	D.F.	S.S.	M.S.S.	F
Extension methods	6	207.15	34.52	27.39**
Education	1	28.42	28.42	22.55**
Interaction	6	50.90	8.48	6.73**
Error	50	63.14	1.26	
Total	63	349.61		

** Significant at 1% level

The significance of difference between each pair of mean conviction scores of respondents exposed to the innovation through different extension methods and their combinations, irrespective of their levels of education, have been shown in Table 2. It is evident from the data that among individual extension methods, the mean conviction score of personal contact was signifi-

Table 2. Mean conviction scores of respondent subjected to different extension methods. The means, which do not differ significantly at 1% level, are underlined

$T_1T_2T_3$	T_1T_2	T_1T_3	T_1	T_2T_3	T_2	T_3
8.0	6.82	6.62	5.38	5.30	3.20	2.33

T_1 = Personal contact; T_2 = Group discussion; T_3 = Bulletin

cantly higher than those of the other two. The difference between the mean conviction scores of group discussion and bulletin was not statistically significant. It is further revealed that when personal contact was associated with group discussion or bulletin, the mean conviction score was significantly higher than that of the combination of group discussion and bulletin. The mean conviction score of the combination of personal contact and group discussion did not differ significantly than the combination of personal contact and bulletin. It was observed that the combination of personal contact, group discussion and bulletin resulted in significantly higher mean conviction scores than any individual extension method or the combination of any two. Thus, among the selected individual extension methods, personal contact was found to be most effective in developing positive conviction about the utility of the innovation. Among the combinations of two, group discussion and bulletin appeared to be least effective. There was no significant difference in the effectiveness of personal contact plus group discussion and personal contact plus bulletin. The combination of personal contact, group discussion and bulletin proved to be most effective than any other individual extension method or other combinations.

Personal contact was found to be the best method to convey new ideas in the local situation. The aquaculturists, in general, considered this source of information dependable. In group discussion, the communication is formal in nature in which the specific farm situations of individual participants are not thoroughly discussed. Similarly, the information provided through bulletin is very general. The potential adopters are sceptical about some printed information because they feel that the information is tempered to cater business interests. It is, therefore, not hard to find why group discussion and bulletin either individually or in combination were not so effective in convincing the respondents about utility of induced breeding technique as personal contact or its combinations with the other two methods could do. The reason why the combination of all three extension methods resulted in most positive conviction is quite obvious. In this case, the respondents were exposed to the same innovation through three different extension methods including personal contact. Naturally, they had greater opportunity to understand the details and dispel their doubts and misgivings about the innovation.

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Fish Consumption Profile of Cochin Households

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Fish forms an integral part of the diet of the non-vegetarian population of Kerala. This paper examines the food consumption profile of selected households in Cochin with reference to different income categories. The average consumption expenditure at mid point of the income classes is more than double the corresponding consumption expenditure for the lowest income group. The total food consumption expenditure tends to plateau off as income increases. The highest four classes of households do not spend more than one-sixth of their income on food consumption. Of the four items of meat, fish, milk and eggs, fish appears to be the most preferred food. The highest three income groups spend almost as much on fish as on staple food. This shows that household income is an important determinant of demand for fish.

Key words: Fish consumption, Cochin, household income

Fish forms a very important source of protein and is an integral part of the diet of non-vegetarian population of Kerala. However, quantity of fish consumed by households is a function of variables such as household income, consumer taste, supply of fish, price of fish and also the supply and price of close substitutes to fish such as meat and poultry products. Fish consumption surveys (BOBP, 1992) constitute an important information input for public policies on marketing, public health and product development. This paper examines the food consumption profile of selected households in Cochin area with a view to brinigng out inter-relations between household consumption of fish and consumption of other food items with reference to different income categories.

Materials and Methods

Food consumption data together with household income data were collected from a sample of 170 households located in different parts of Cochin city (Kerala, India). The households have been classified into eleven income groups and the consumption of different food items have been profiled on the basis of average consumption per household. Food items accounted for in this study are staple food, fish and animal products (meat, milk, eggs)

Results and Discussion

Table 1 presents food consumption profile of households classified into eleven income groups. Mean household expenditure on foods ranged from Rs.27.7 to Rs.108.4 per day among different income groups. At midpoint of income classes (Rs.251-300) average household expenditure on foods was Rs.62. This was more than double the value obtained for lowest income group. Household expenditure on foods increased steadily from Rs.27.7 to 78.3, for income classes <50 to 301-400. Thereafter, it fluctuated between Rs.49.9 and 108.4 (Table 1). Mean per capita expenditure on foods showed a similar trend.

Food consumption expenditure as percentage of income is given in Tab.1. The trend followed Engels' law (Paul & Nordhaus, 1995), which stated that food consumption expenditure as percentage of income, tend to fall as the income increases. Food consumption expenditure was 88% of the income for poorest households. The very next class had a consumption expenditure of only 47% of the income and this class-wise decrease in the percentage continued steadily up to the

Table 1. Family size, per capita expenditure on food, household income and food consumption expenditure

Income class, Rs.	No. of respondents	Average family size	Per capita expenditure on food (Rs./Day) Mean±SD	Household income (Rs./Day) Mean±SD	Household food consumption expenditure (Rs./Day) Mean±SD	Food consumption expenditure as % of income
0-50	19	3	7.83±2.45	31.29±14.63	27.67±16.78	88.41
51-100	28	4	10.02±4.06	81.03±16.70	37.95±16.59	46.83
101-150	27	4	11.30±4.34	131.86±14.32	43.10±11.53	32.68
151-200	29	5	13.49±6.01	179.72±16.55	59.77±20.84	33.26
201-250	7	6	10.95±5.08	230.95±6.30	58.46±36.27	25.31
251-300	23	5	14.86±8.87	278.26±15.44	61.57±29.66	22.13
301-350	16	5	16.26±6.13	333.33±0.00	78.28±37.64	23.48
351-400	3	6	9.30±2.74	388.89±19.25	49.92±22.51	12.84
401-450	2	3	24.36±16.22	433.33±0.00	73.07±48.66	16.86
451-500	10	6	15.09±10.78	496.67±10.54	70.87±27.43	14.27
>500	6	6	18.90±6.49	733.33±101.11	108.43±41.46	14.79

income class of Rs.301-350. Thereafter, it was below 20%. The highest four classes of households not spend more than one-sixth of their income on food consumption.

A notable feature of mean food consumption expenditure for the various income groups was the high standard deviation around mean. Notably, standard deviation representing the inter-family variation in food expenditure for the same income group was relatively lower in the lower income groups than in the higher income groups. This does not mean that there is more homogeneity in the consumption profile of lower income groups. A lower standard deviation can be attributed to income imposed squeeze on consumption on poorer families.

The relationship between income and food expenditure is established through regression statistics as shown in Table 2. The linear regression equation derived was $Y = 10.34 + 0.21X$, where Y is household food consumption expenditure and X is the household income. The correlation between income and expenditure was 0.55, reflecting that though the relation between income and expenditure was not very high, it was fairly well established and R^2 value was found to be 0.3. It showed that only 30% of the factors responsible for food consumption expenditure was explained by income. This is highly reasonable since income alone cannot explain household food consumption. Factors such as age distribution of the families, the size of the families, the household's propensity to spend on non-food items such as cloth, housing facilities, entertainment also determine money spent on food consumption. The R^2 value of 0.3 can therefore be treated as a reasonable fit.

Table 2. Regression between household income and food consumption expenditure

Multiple R	0.55
R^2	0.30
Adjusted R^2	0.30
Standard error	25.17
Observations	170.00

Food expenditure has been classified into expenditure on staple food, fish, meat, milk and egg. Expenditure on staple food ranged between Rs.16 and Rs.36 between the lowest and the highest income groups. It is to be noted that this expenditure did not truly reflect the expenditure on food at market prices. Every household draws a part of the food requirement from the public

distribution system. This component of expenditure does not depend upon income because quantity of rationed food like wheat and rice is fixed on the basis of family size and not on the basis of income. Of course, at very low levels of income, households get more subsidy on food in the form of reduced prices on ration rice, thereby meeting a part of the consumption expenditure from government subsidy. The point to be stressed is that expenditure on staple food would have been considerably higher if this component of subsidy was absent. There was a steady increase in the expenditure on staple food consumption. This was Rs.36 for the highest income group and Rs.16 for the lowest income group indicating the rather stable relation between income and staple food. Compared to this, the expenditure on other food items such as fish, meat, milk and egg did not exhibit a pattern. A considerable element of inter class variation was observed in the consumption of these four items.

Of the four items of meat, fish, milk and egg, fish appeared to be the most prefered quality food and egg constituted the least favored as may be seen from Table 3. Even for those households that were in the lowest income group, mean expenditure on fish is Rs.3 per day whereas no class of households had expenditure on egg exceeding a mean of Rs.2.65. The sample households had therefore a strong preference for fish in comparison to egg.

Expenditure on fish consumption as opposed to that on staple food clearly falls into two patterns. The highest three income groups spent almost as much on fish as on staple food. This showed that household income was an important determinant of demand for fish. Higher the income, higher will be the percentage of the component of fish in the daily diet chart. Clearly, fish product development should aim at capturing the fancy of high income groups if more monetary return per quantity of raw fish is to be realized.

When compared to fish, meat products did not constitute a very significant item of household consumption. Average expenditure on meat exceeded Rs.10 only for the highest three classes of income. It was reported during the survey that fish was consumed in small quantities most of the days while meat was a luxury consumed only on occasions. Therefore, it can be safely asserted that frequency of fish consumption is more than the frequency of meat consumption. This was reflected in the mean

Table 3. Average food consumption expenditure on various items

Income class	Average food consumption expenditure (Rs./day)				
	Fish	Staple food	Meat	Milk	Eggs
0-50	2.93±2.7	16.12±10.16	2.60±3.76	5.63±6.10	0.38±0.41
51-00	10.13±7.80	17.10±7.68	5.26±3.76	4.95±3.98	0.51±0.88
101-150	11.57±7.43	20.39±8.59	4.19±2.82	6.06±4.93	0.88±0.95
151-200	17.14±18.97	23.59±10.67	7.23±4.09	10.42±5.15	1.38±1.19
201-250	15.96±13.12	25.14±16.62	5.26±4.30	10.63±4.98	1.38±1.83
251-300	16.84±27.80	26.88±10.18	6.40±6.79	9.73±8.27	1.72±1.97
301-350	25.13±20.89	27.85±17.02	9.46±6.29	14.42±8.85	1.40±1.83
351-400	11.67±8.33	26.79±14.59	4.29±1.43	6.64±0.56	0.54±0.54
401-450	25.83±27.11	23.50±5.42	10.71±9.09	12.48±7.80	0.54±0.76
451-500	23.16±19.66	25.44±10.72	10.07±10.93	10.44±5.70	1.76±2.18
>500	26.14±19.04	35.57±7.93	23.21±22.57	20.86±11.52	2.65±1.18

consumption pattern of households. Milk consumption in terms of household preference fell midway between fish and meat consumption. As with meat, milk consumption also accounted for less than Rs.21 per household per day showing a very insignificant pattern with reference to income. It appears that the milk consumption is not related to income in any recognizable pattern but depends mostly on the age structure of the household. Larger the number of children, higher was the milk consumption, irrespective of the income.

The study clearly showed that fish occupied a place next only to staple food in the household preferences of the Cochin area. It had shown a positive relation with household income. No other quality food such as meat, milk and egg had such a positive association with income. Fish was the most important source of protein for all classes of population.

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(b) Books/approved methods

i) Brandt, A.V. (1984) *Fish Catching Methods of the World*, 3rd edn., p. 253, Fishing News Books, Farnham, UK

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(c) Chapters in edited books/papers in symposium proceedings

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Rajyasree M. & Neeraja, P., (1993) Paper 48 presented at the Int. Symp. on Nutrients and Bioactive Substances in Aquatic Organisms (SOFT (I) & CIFT) Cochin, India, 14-17 Sept.

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